MUTUAL IMPACTS IN A SPECIALIST HERBIVORE AND ITS HOST PLANTS: VARIATION IN INSECT MORPHOLOGY AND PLANT TOLERANCE

A Thesis

by

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ABSTRACT

In one study, a suite of host plants from the genus *Zea* L. (Poaceae) and the specialist herbivore *Dalbulus maidis* (DeLong and Wolcott) (Hemiptera: Cicadellidae) were used to address whether plant tolerance to direct damage by the herbivore and seedling morphometry were mediated by plant domestication and genetic improvement. Additionally, the role of shoot: root ratios in plant tolerance was included in this study. Plant tolerance was measured as regrowth rate, and a trade-off between plant tolerance and resistance was predicted based on (i) the increasing investment in growth and productivity with evolutionary history in *Zea* and, (ii) its negative correlation with plant resistance. The effects of the domestication transition were assessed by contrasting Balsas teosinte (*Z. mays* L. ssp. *parviglumis* Iltis & Doebley) and maize (*Z. mays* L. ssp. *mays*), while breeding transition by contrasting maize landraces and maize inbred lines. The results showed that domestication and breeding mediated changes in seedling morphometry, but did not mediate changes in plant tolerance, with Balsas teosinte, maize landraces, and maize inbred lines similarly tolerant to feeding damage by *D. maidis*. In contrast, domestication mediated changes in shoot: root ratios, with larger roots in the maizes, suggesting increased storage capability in domesticated taxa.

In another study, morphometrics analyses were performed on *D. maidis* specimens collected from Perennial teosinte [*Zea diploperennis* Iltis (Doebley & Guzman)] and maize in western Mexico, to determine whether a correlation existed between previously documented genetic differentiation and morphological

(morphometrical) differentiation. Traditional morphometrics was used to analyze body differences, and geometric morphometrics was applied for wing analysis. The results suggested that genetic and morphological differentiation are correlated in *D. maidis*, with individuals associated to Perennial teosinte exhibiting larger body size compared to individuals associated to maize. A strong host plant effect and a moderate habitat effect were detected, and body size was found to be as the variable explaining most of the variance between individuals. Wing differentiation between individuals associated with Perennial teosinte and maize was detected, but it was strongly correlated with allometry. It was hypothesized that different selection forces are acting on morphological and genetic differentiation because while morphological differentiation is maintained in locations where Perennial teosinte and maize coexist, genetic differentiation is lost in those locations. Overall, the study's results suggested that evolutionary transitions in *Zea* have exerted changes in both the plant morphometry and insect morphology, with changes in the plant related to increased storage and productivity in roots, and changes in the insect related to overall body size.

DEDICATION

To my parents, Rolando and Teresa, and to Nathaniel.

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CHAPTER I

INTRODUCTION

Interactions between plants and insects are among the closest and most dynamic ecological relationships in nature, with both taxa exerting mutual effects on one another. Feeding and oviposition by herbivorous insects can effect changes in plants at different levels; for example, induced responses after herbivory attacks have been reported in more than 100 plant-herbivore systems, with increased levels of physical, chemical and biotic defenses in plants subjected to insect damage (Agrawal 1998), as well as changes in plant volatile emissions after egg deposition by herbivores (Hilker and Meiners 2011). Moreover, Price et al. (2011) suggested that insects can alter the source-sink dynamic within plants by diverting assimilates from neighboring leaves to the feeding site to enhance their performance, and also argue that herbivory is one of the most important forces explaining the existence of secondary compounds in plants. Conversely, insects also respond to plant stimuli modifying their behavior and/or metabolism to adapt to novel conditions in their hosts; for instance, detoxification, excretion, sequestration, behavioral deactivation or avoidance are mechanisms displayed by different species to cope with plant defenses (Price et al. 2011). Importantly, variation in morphology has also been reported as a response to different host plants; for example, Jorge et al. (2011) showed variation in wing size and shape in the butterfly *Heliconius erato* L. among individuals feeding on different host plants. Furthermore, the environmental conditions in which plants grow can also partially mediate variation in herbivore morphology; for

instance, Barman (2011) showed that host plants and geographical distribution play important roles in the morphological variation found in the wings of *Pseudatomoscelis seriatus* (Reuter).

Evolutionary and anthropogenic forces also mediate variation in plant and herbivore traits. In one study, Rosenthal and Dirzo (1997) showed that evolutionary and human selection processes leading towards higher productivity in the genus *Zea* L. lead to a gradual weakening of plant defense mechanisms. This was confirmed in a posterior study examining the performance of the specialist herbivore *Dalbulus maidis* (DeLong and Wolcott) on maize and its wild relatives; the performance of the herbivore was superior in plants selected for higher yields, due to the presumably weaker defenses compared with low-yielding wild cultivars, with presumably stronger defenses (Dávila-Flores et al. 2013). Another study encompassing the same suite of host plants and specialist herbivore showed that physical traits were also affected by the same evolutionary and human selection transitions in that leaf toughness decreased and *D. maidis* oviposition increased with increasing plant productivity (Bellota et al. 2013).

The goal of this study was to address in part the mutual impacts between the plant genus *Zea* (Poaceae) and the specialist herbivore *Dalbulus maidis* (DeLong and Wolcott) (Hemiptera: Cicadellidae) in the context of evolutionary- and anthropogenic-driven processes, such as domestication and breeding. Chapter II addresses whether plant tolerance of feeding by *D. maidis* is mediated by *Zea* domestication, a process transforming a low-productivity wild annual to intermediate-productivity domesticated annual, and maize breeding, a process transforming an intermediate-productivity

domesticated annual to a high-productivity domestic. Chapter III addresses whether morphological differentiation is consistent with previously documented genetic differentiation in *D. maidis* associated with different *Zea* host plants. This study contributes to our understanding of how evolutionary and anthropogenic forces may mediate both plant defense mechanisms (i.e., tolerance, Chapter II) and morphological variation in insects (i.e., morphological differentiation, Chapter III), in so doing it enhances our understanding of ecological and evolutionary processes shaping pest contexts in contemporary agriculture.

CHAPTER II

SEEDLING MORPHOMETRY BUT NOT HERBIVORY TOLERANCE IS MEDIATED BY MAIZE DOMESTICATION AND BREEDING

Introduction

Interactions between plants and insects can vary from beneficial (e.g., pollination) to detrimental (e.g., herbivory). Detrimental interactions, such as herbivory, have been widely studied from both the herbivore and the plant perspectives, giving rise to different hypotheses concerning the way insects may cope with plant defenses, and the mechanisms plants may use to prevent and overcome herbivory. Plants have evolved a variety of strategies to cope with herbivores and pathogens, including avoidance in time and space, resistance—i.e. any chemical or physical trait in the plant that reduces preference or performance of herbivores—, and tolerance—i.e. the degree to which plant fitness is affected by herbivory relative to fitness in undamaged plant (Belsky et al. 1993; Strauss and Agrawal 1999). While tolerance is usually studied in the context of herbivory by vertebrate and invertebrate organisms, some studies suggest that other stresses, such as fires, droughts, and frosts may also contribute to the development of tolerance as a plant response for overcoming damage and persist in the environment (Belsky et al. 1993; Rosenthal and Kotanen 1994). Strauss and Agrawal (1999) found that the most common approach to measuring tolerance is through compensation, which is defined as the degree of re-growth exhibited by a plant following herbivory; when related damaged and undamaged plants have the same fitness they are considered fully

tolerant, whereas if damaged plants show lower or higher fitness they are considered to under-compensate or overcompensate, respectively. Additionally, Strauss and Agrawal (1999) indicated that various other mechanisms may also be associated to increased tolerance, e.g., increases in net photosynthetic and relative growth rates, increases in branching or tillering after release of apical dominance, pre-existing high levels of carbon storage in roots for allocation to above-ground reproduction, and relocation of carbon stores from root to shoot after herbivory. In particular, interest in the role of roots in above-ground defense has increased in recent years, with several studies calling for a more systemic approach to studies of plant defense in which above-ground and below-ground processes in the plant are linked for a better understanding of plant-herbivore interactions (Kaplan et al. 2008; Erb et al. 2009; Nalam et al. 2013, Soler et al. 2013).

Several plant defense hypotheses suggest a fitness cost associated with defense, hence a dilemma faced by plants: whether they should grow fast enough to compete, or maintain physiological adaptations–i.e. defenses–necessary for survival in the presence of herbivores and pathogens (Herms and Mattson 1992; Stamp 2003). Thus, the growth-differentiation balance hypothesis predicts that allocation of resources by plants to chemical and structural defenses decreases growth by diverting resources from the production of leaf area and other vegetative structures (Herms and Mattson 1992). At least three hypotheses have been proposed on the basis of a presumed trade-off between primary metabolism (growth and reproduction) and secondary metabolism (i.e. defense): plant species will either have well-developed tolerance and weak resistance mechanisms, poor tolerance and well-developed resistance mechanisms, or intermediates of both

mechanisms (van der Meijden et al. 1988). For example, Fineblum and Rausher (1995) reported that morning glory (*Ipomoea purpurea* L. Roth) genotypes with higher defense levels against insects causing apical damage showed lower tolerance to the same type of damage. In contrast, positive associations between these two defensive mechanisms have been reported, and some evidence indicates that tolerance and resistance can be alternative strategies, co-occurring together in the same plant (Rosenthal and Kotanen 1994; Mauricio 2000; de Mazancourt et al. 2001). Other studies suggest that tolerance is a strategy developed by plants in response to specialist herbivores given that these insects employ diverse mechanisms to attenuate or overcome negative impacts of defenses, such as trichomes, latex, and secondary compounds (Agrawal and Fishbein 2008).

Another plant defense hypothesis relevant to the study of tolerance considers the availability of resources in the environment in which a plant species evolves (Coley et al. 1985). That hypothesis predicts that plant species evolving in resource-rich environments (e.g., domesticated plants) exhibit faster growth and leaf turnover rates, and greater tolerance, whereas species evolving in resource-poor environments grow slower, but have superior defense systems (e.g., wild taxa) (Lind et al. 2013). Within that framework, crop plants and their wild relatives are a useful model for studying the evolution of tolerance because domestication and genetic improvement through breeding strongly select for plant traits relevant to increased growth and reproduction rather than defense, consistent with the hypothesis that plant productivity and defense are negatively correlated. For example, in a study encompassing the maize genus *Zea* (Poaceae),

Rosenthal and Dirzo (1997) found evidence of differential investment in growth and defense against herbivores in maize and its wild relatives, with wild taxa growing slower, but with better defenses against insects compared to landrace and modern maize cultivars. Moreover, plant productivity increased gradually with life history evolution (from perennial to annual life history), domestication (wild annual to domesticated annual), and genetic improvement (selection by farmers to systematic breeding), whereas the opposing trend was evident for herbivore defenses, which became weaker with those processes. Similarly, Dávila-Flores et al. (2013) compared the performance of the specialist, sap-sucking herbivore corn leafhopper [*Dalbulus maidis* (Delong & Wolcott)] (Hemiptera: Cicadellidae) on a suite of *Zea* spp. representing the plant genus' evolution from wild perennial taxa to wild annual taxa to domesticated and increasingly improved maize cultivars, and found that the leafhopper's performance increased from the wild perennial taxon through the most-improved maize cultivar, consistent with the results of Rosenthal and Dirzo (1997).

In this study, I addressed whether plant tolerance to direct damage by a specialist herbivore and seedling morphology are mediated by plant domestication and improvement. To that end, I assessed tolerance to corn leafhopper within a suite of *Zea* spp. host plants encompassing those two transitions, domestication and improvement. The domestication transition was represented by Balsas teosinte (*Z. mays* L. ssp. *parviglumis* Iltis & Doebley) and maize (*Z. mays* L. ssp. *mays*), and the breeding transition by maize landraces and maize inbred lines. Balsas teosinte is the immediate ancestor of maize, and maize landraces are the predecessors of inbred lines. Overall, I

expected to find evidence that domestication and breeding in *Zea* (i) selected for increasingly higher tolerance (i.e. decreased fitness cost to plant under herbivory), while selecting for increasingly lower resistance and greater productivity (Rosenthal and Dirzo 1997, Bellota et al. 2013, Dávila-Flores et al. 2013), and (ii) mediated seedling morphology. Also, root/shoot ratios were compared across transitions, and discussed in the context of domestication and breeding to explore the role of below-ground tissues in tolerance to herbivory. Of particular importance in this study is the analysis of tolerance responses to damage by a sap-sucking insect in contrast to tolerance studies carried out with chewing insects.

Methods

Zea *and* Dalbulus maidis

The genus *Zea* L. (Poaceae) is native to Mexico and Central America, and includes five species: *Zea diploperennis* Iltis, Doebley & Guzman, *Z. perennis* (Hitchcock) Reeves & Mangelsdorf, *Z. luxurians* (Durieu & Ascherson) Bird, *Z. nicaraguensis* Iltis & Benz, and *Zea mays* L, which includes four subspecies: *Z. mays* L. ssp. *huehuetenangensis* (Iltis & Doebley) Doebley, *Z. mays* L. ssp. *mexicana* (Schrader) Iltis, Balsas teosinte, and maize (Buckler & Stevens 2005). Archeological and molecular evidence confirm that maize was domesticated from Balsas teosinte ca. 9200 years ago in western Mexico (Matsuoka et al. 2002). This study evaluated tolerance in Balsas teosinte and maize, as noted above.

Leafhoppers in the genus *Dalbulus* (DeLong) are specialists on grasses (Poaceae), particularly in the genera *Tripsacum* L. and *Zea*. The genus' center of diversity and speciation broadly overlaps with that of *Zea*, which has brought special attention to the genus, and several studies suggest that it coevolved with *Zea* in Mexico (Nault and Delong 1980; Triplehorn and Nault 1985; Dietrich et al. 1998). Indeed, it was suggested that corn leafhopper followed the expansion pattern of maize after its domestication from Balsas teosinte (Nault 1990, Medina et al. 2012) The corn leafhopper is widely distributed in the Americas, and has been found as far north and south, respectively, as Ohio in the United States and northern Argentina in South America, as well as in the Caribbean (Nault and Delong 1980; Summers et al. 2004; Medina et al. 2012).

Study system: insects and host plants

A laboratory culture of corn leafhopper was established with insects collected with sweep nets and aspirators from landrace maize in the vicinity of El Grullo (Jalisco state, Mexico; 19°48'N, 104°13' W) in the summer of 2008. The culture was kept in a plastic frame mesh cage (BugDorm-44545F, Megaview Science Co., Ltd., Taichung 40762, Taiwan) on seedlings of a Mexican landrace of maize (Elotes Occidentales), usually in their 4-6 leaf stage, in a room with a photoperiod of 12:12 (L:D) and a temperature of 24-28 °C. Uniformly-aged (7-14 days old ± 2 days) corn leafhopper males were obtained by placing landrace maize seedlings inside the cage holding the corn leafhopper culture for 4 days, and then moving the exposed seedlings to empty

collapsible cages (Collapsible observation and rearing cage-1466A, Bioquip Products, California 90220, USA) after removing all corn leafhopper nymphs and adults from the seedlings. Eggs laid in the seedlings were allowed to hatch, and individuals that developed to adults were kept in the cages until used in the experiment. This process was repeated weekly to maintain a constant source of male corn leafhoppers.

Host plants used in this experiment consisted of a suite of nine different Balsas teosinte or maize accessions: three each, Balsas teosinte, maize landrace, and maize inbred line accessions. Seed of Balsas teosintes accessions were collected in Guachinango (Guachinango municipality, Jalisco state, Mexico; 20°46′N, 104°05′W), San Lorenzo (Ejutla, Jalisco, Mexico; 19°56’60”N, 103°59’0” W) and El Cuyotomate (Ejutla, Jalisco, Mexico; 19°58’10.39”N, 104°4’3.00”W). Landrace maize accessions included Tuxpeño landrace, which was obtained from USDA NPGS (GRIN accession PI 511649), and Tepecintle and Oloton landraces which were obtained from Fidel Márquez Sánchez (CRUOC, Universidad Autónoma de Chapingo). Seeds of maize inbred line accessions B73 and CML176 were obtained from Michael Kolomiets (Plant Pathology and Microbiology, Texas A&M Uniersity), and of inbred line accession MP708 from USDA NPGS (GRIN accession PI 536520).

All seeds were germinated in the laboratory using BACCTO Premium Potting soil (85-15-10) (Michigan Peat Company, Houston, TX, USA). Seeds of all host plants were placed in Petri dishes with heat-sterilized, sieved soil, covered with a paper towel, and watered as necessary. A nail clipper was used to remove the fruit-case covering the Balsas teosinte seeds. After germination, seedlings were transplanted to cone-tainers (4

cm diam. x 25 cm length) (Stuewe & Sons, Tangent, Oregon, USA) and placed inside rectangular collapsible cages (Collapsible observation and rearing cage-1466A, Bioquip Products, California 90220, USA) to prevent accidental contamination with corn leafhoppers. Seedlings were watered every 4-5 days. Germination and growth of the seedlings were carried out in a room with a photoperiod of 12:12 (L:D) and a temperature of 28-32 °C.

Herbivory tolerance experiment

Seedlings from each host plant in stages V4-V5 (= 4-5 collared leaves) were selected, and pairs of seedlings of similar size (per visual assessment) and equal number of leaves were created. Posteriorly, seedlings were placed inside transparent tubular plastic cages (35 cm x 9 cm diam.); one opening of the cage was attached to the cone-tainer with adhesive tape, and the upper opening was covered with an elastic mesh to allow air circulation and to contain corn leafhoppers during the experiment. One seedling of every pair was randomly selected to be infested with five male corn leafhoppers, while the other seedling was used as an un-infested control. Males were exclusively used in the experiment to maintain a constant number of corn leafhoppers on the seedlings during the experiment. An aspirator was used to transfer corn leafhopper males from the collapsible cages to the tubular plastic cages. Infested seedlings were examined daily, and dead corn leafhoppers were replaced as needed. Infested and control caged seedlings were kept during 14 days in a room with a photoperiod of 12:12 (L:D) and a temperature of 28-32 °C; seedlings were watered every 4-5 days by immersing the

bottom half of the cone-tainers in water for 2 hours. After 14 days, corn leafhoppers were removed and the seedlings were extracted from the cages and harvested in order to record measurements from the root and the foliage. After excising the root from the stem, large soil particles attached to the roots were carefully removed by hand, and smaller particles were removed by soaking and rinsing the roots in tap water. Washed roots were blot dried by pressing them between paper towels three times to remove excess water. The following variables were measured in the above-ground portion of the seedlings: total length of the uppermost four leaves (exclusive of whorl leaves; measured from the leaf collar to the leaf tip), average stem diameter (average of three measurements taken between the whorl leaf and the first collared leaf, using a digital micrometer Pittsburgh-68305, Harbor Freight Tools, California, USA), wet weight (measured immediately after excising the roots), and dry weight (measured after drying in an oven at 80 °C for $\geq$ 3 days). For the roots, the following variables were measured: total length (from the excision point to the root tip), wet weight (measured immediately after washing and blot-drying the roots), and dry weight. A total of 34 Balsas teosinte seedling pairs (Guachinango = 13, San Lorenzo = 11, El Cuyotomate = 10), 47 landrace maize pairs (Tuxpeño = 16 , Tepecintle = 15, Olotón = 16), and 44 maize inbred line pairs (B73 =16, CML176 = 15, MP708 = 13) were evaluated as described above.

Statistical analyses

I performed a full factorial MANOVA to assess whether herbivory by Corn leafhopper affected seedling architecture (= shape) and form (= architecture + size)

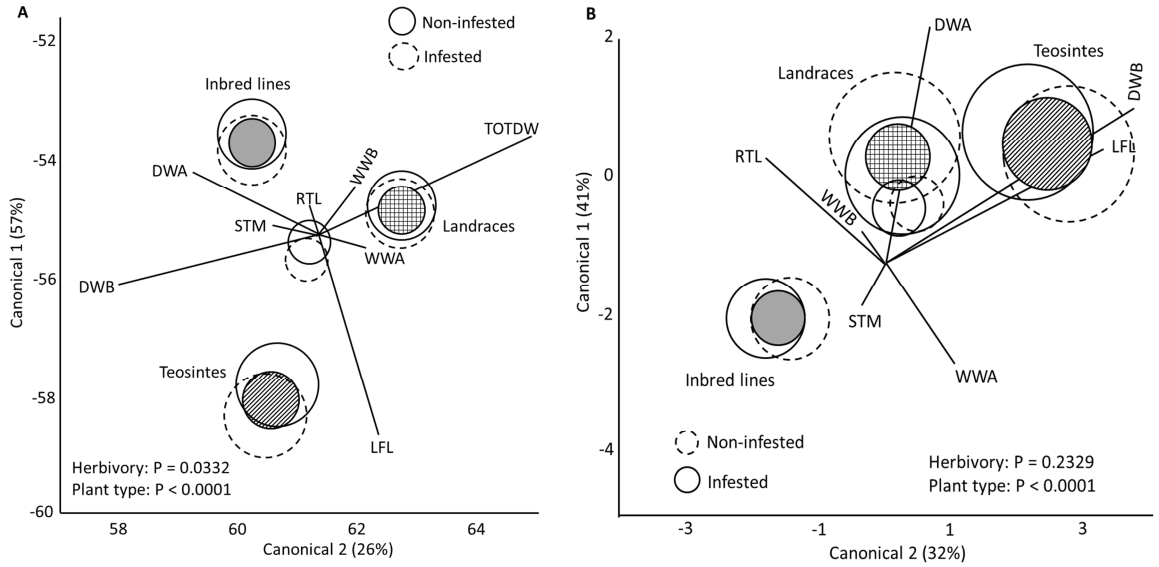
across the three plant types and across three accessions within each plant type. Specifically, MANOVA included the independent variables “Herbivory” (infested, un-infested), “Plant type” (Balsas teosinte, maize landrace, maize inbred line), and “Accession” (three per Plant type) nested within Plant type, and all interactions among them, while the dependent, response variables were the above-ground and root variables described above (above-ground: leaf length, average stem diameter, wet weight, and dry weight; roots: total root length, wet weight, and dry weight). Prior to analyses, all non-linear, response variable data were linearized; once linearized, all data were size-scaled using seedling total dry weight (= above-ground dry weight + root dry weight), and transformed to their natural logarithmic values to meet normal distribution assumptions. The seedling architecture analysis included total dry weight as an independent variable, as well as all possible interactions with the balance of independent variables (Herbivory, Plant type, Accession). The seedling form analysis included total dry weight as a response variable, and considered a one-tailed P value for the effect of Herbivory because seedlings infested with corn leafhopper were expected to be smaller than un-infested seedlings. Planned, *a priori* contrasts (with Sidak’s multiple comparisons correction) (Abdi and Williams 2010) were used to assess the effects, if any, of domestication (Balsas teosintes vs. maize landraces) and breeding (maize landraces vs. maize inbred lines) on seedling form and architecture. Correlations on canonical scores were used to determine the contribution of each independent variable (Pearson’s r^2) to variation in the first two canonical axes of MANOVA centroid plots; only correlations > 0.50 and $P < 0.05$ were considered in Results.

I applied ANOVA to determine whether the ratios of above-ground weight to root weights (both dry and wet weights) were mediated by Herbivory, Plant type, and Accession nested with Plant type. Prior to analysis, above-ground to root ratios were transformed to their natural logarithmic values to meet normal distribution assumptions. Planned, *a priori* contrasts were used to assess the effects, if any, of domestication and breeding on seedling above-ground weight to root weight ratios, as described above.

Results

MANOVA showed a significant multivariate effect on seedling form (i.e. architecture + size) (Wilks' $\lambda = 0.032$, $P < 0.0001$) (Fig. 2.1a). The main effect of Herbivory on plant form was significant across plant types (one-tailed $P = 0.033$), and, while the Plant type effect was significant ($P < 0.0001$), the Herbivory × Plant type interaction was not significant ($P = 0.713$). Planned, *a priori* contrasts for Plant type effect revealed significant effects of the domestication ($P < 0.0001$) and breeding ($P < 0.0001$) transitions on seedling form (Fig. 2.1c). The vertical axis in the canonical centroid plot explained 57% of the variation, with leaf length (LFL) as the variable contributing the most to segregation of teosintes from the two maizes ($P < 0.05$, $R^2 = -0.89$), whereas the horizontal axis explained 26% of the variation, with total dry weight (TOTDW) ($P < 0.05$, $R^2 = 0.79$) and root length (RTL) ($P < 0.05$, $R^2 = -0.70$) as the

variables segregating maize landraces from teosinte and maize inbred lines. MANOVA on seedling architecture showed a significant multivariate effect (Wilks' $\lambda = 0.003$, $P < 0.0001$) (Fig. 2.1b). The main effect of Herbivory was not significant (one-tailed $P = 0.233$), while Plant type was significant ($P < 0.0001$), but its interaction with Herbivory was not significant ($P = 0.128$). Planned, *a priori* contrasts for Plant type revealed significant effects of the domestication ($P < 0.0001$) and breeding ($P < 0.0001$) transitions on seedling architecture (Fig. 2.1d). The vertical axis in the canonical centroid plot explained 41% of variation, with leaf length (LFL) ($P < 0.05$, $R^2 = 0.76$), root length (RTL) ($P < 0.05$, $R^2 = 0.72$), above-ground dry weight (DWA) ($P < 0.05$, $R^2 = 0.63$), below-ground dry weight (DWB) ($P < 0.05$, $R^2 = -0.65$) as the variables segregating maize inbred lines from teosintes and maize landraces; the horizontal axis explained 32% of the variation, with leaf length (LFL) ($P < 0.05$, $R^2 = 0.55$) and root length (RTL) ($P < 0.05$, $R^2 = -0.54$) as the variables segregating maize inbred lines from maize landraces and teosintes.



C

Contrast	$F_{8, 223}$	P
Domestication (Balsas teosinte vs. maize landrace)	70.066	< 0.0001
Breeding (maize landraces vs. maize inbred)	42.812	< 0.0001

D

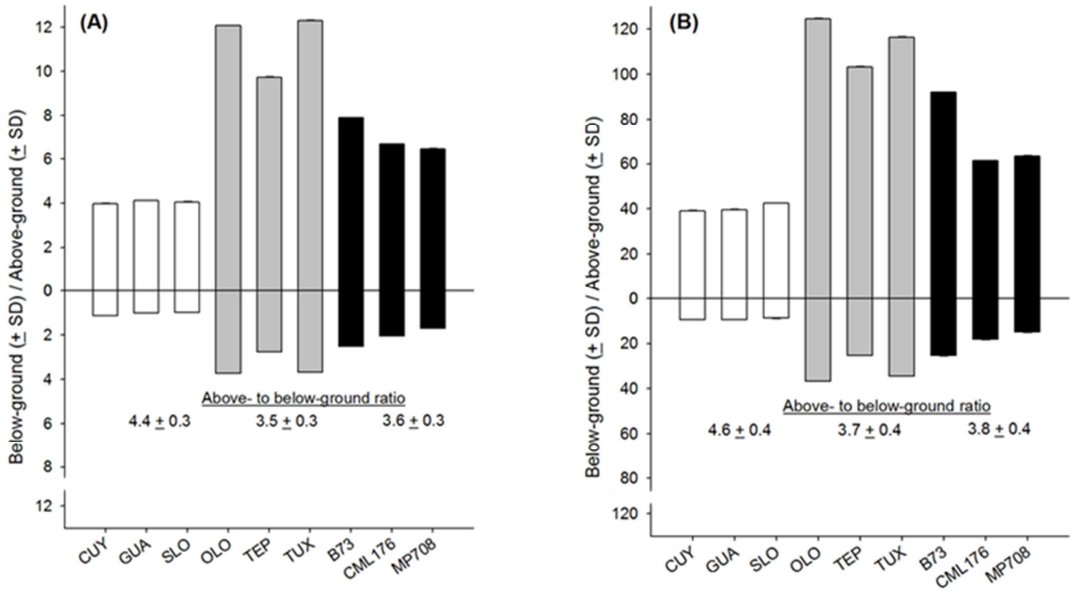
Contrast	$F_{7, 207}$	P
Domestication (Balsas teosinte vs. maize landrace)	15.714	< 0.0001
Breeding (maize landraces vs. maize inbred)	38.007	< 0.0001

Figure 2.1 Canonical centroid plots for seedling form and architecture. Overall, MANOVA showed that Herbivory (infested with corn leafhopper, non-infested), Plant type (Balsas teosinte, maize landrace, maize inbred line) and Accession (three per Plant type, nested within Plant type) affected seedling form **(A)** and architecture **(B)** in Balsas teosinte, maize landraces and maize inbred lines accessions. **A)** MANOVA on plant form (size + shape) showing significant difference between infested and non-infested plants (Wilks' $\lambda = 0.032$, $P < 0.001$, $F = 7.242$, df = 136, 1638.8); Herbivory significantly affected seedling form across all accessions ($P = 0.03$), but the effect was independent of Plant type and Accession. **B)** MANOVA on plant architecture (i.e. size excluded) showing no significant difference between infested and non-infested plants (Wilks' $\lambda = 0.003$, $P < 0.001$, $F = 7.750$, df = 238, 1434.7); Herbivory did not affect seedling architecture across all accession ($P = 0.233$) and it had no interaction with Plant type or Accession. **C)** Planned, *a priori* contrasts for Plant type showing significant effect of domestication and breeding on plant form. **D)** Planned, *a priori* contrasts for Plant type showing significant effects of domestication and breeding on plant architecture. Filled circles represent mean values for each Plant type. Mean values for infested plants are circled with dashed lines; mean values for non-infested plants are circled with solid lines. DWA: dry weight above-ground; RTL: root length; WWB: wet weight below-ground; TOTDW: total dry weight; WWA: wet weight above-ground; LFL: leaf length; DWB: dry weight below-ground; STM: stem diameter.

ANOVA on dry weight showed differences in shoot: root ratios across plant types (P = 0.029) (Fig. 2.2a). Plant type effect was significant (P = 0.0003), whereas Herbivory and Accession effects were not significant (P = 0.870 and P = 0.220, respectively). Planned, *a priori* contrasts on Plant type revealed a significant effect of the domestication transition (P = 0.0002), but a non-significant effect of breeding transition (P = 0.686) (Fig 2.2c). Similarly, ANOVA on wet weight revealed differences in shoot: root ratios across plant types (P = 0.042) (Fig. 2.2b). Plant type effect was significant (P = 0.001), whereas Herbivory and Accession effects were not significant (P = 0.849 and P = 0.070, respectively). Planned, *a priori* contrasts on Plant type revealed that the domestication transition was significant (P = 0.001), while the breeding transition was not (P = 0.637) (Fig. 2.2d).

Discussion

Overall, my results suggested that plant tolerance to corn leafhopper feeding damage was not affected by two important transitions in the genus *Zea*: domestication (wild annual vs landrace) and breeding (landrace vs inbred line), as indicated by the non-significant Plant type by Herbivory interaction (P = 0.713). Although the main effect of Herbivory was significant (P = 0.033) in the overall analysis, its significance was probably influenced by the multiple independent variables combined so that differences became non-significant when the sample size was reduced to analyze the interactions between Herbivory and each Plant type separately. A significant Plant type effect,



(C)

Contrast	$F_{1,230}$	P
Domestication Teosinte vs. Landrace	14.7021	0.0002
Breeding Landrace vs. Inbred line	0.1644	0.6855

(D)

Contrast	$F_{1,230}$	P
Domestication Teosinte vs. Landrace	12.2192	0.0006
Breeding Landrace vs. Inbred line	0.2230	0.6372

Figure 2.2 Above- to below-ground ratios for dry and wet mass. Overall, ANOVA showed a significant effect of the domestication transition and a non-significant effect of the breeding transition on shoot: root ratio, with an increased root size in the maizes (landraces and inbred lines) compared to teosintes. **A)** total dry matter shoot: root ratios; **B)** total wet matter root/shoot ratios for the teosinte and maize accessions tested for tolerance to herbivory by *Dalbulus maidis*; **C)** Planned contrasts on dry shoot: root ratios showing significant difference in dry mass ratios between teosinte and maize landrace (domestication transition) and no significant difference between maize landrace and maize inbred line (breeding transition); **D)** Planned contrasts on wet shoot: root ratios showing significant difference in mass ratios between teosinte and maize landrace (domestication transition) and no significant difference between maize landrace and maize inbred line (breeding transition). White bars are teosinte accessions, grey bars are landrace accessions, and black bars are inbred line accessions. CUY: Cuyotomate; GUA: Guachinango; SLO: San Lorenzo; OLO: Olotón; TEP: Tepecintle; TUX: Tuxpeño**.**

but a non-significant effect of Herbivory × Plant type interaction on seedling form (Fig. 2.1a) indicated that differences between Plant types were not due to corn leafhopper feeding injury, but to the differences among Plant types; this was confirmed with the analysis on plant architecture, which showed a significant Plant type effect ($P < 0.001$), though its interaction with Herbivory was not significant ($P = 0.128$) (Fig. 2.1b). These results are inconsistent with the expectation of a trade-off between tolerance and resistance in the context of the growth-differentiation balance hypothesis generally (Herms and Mattson 1992), as well as specifically in the case of *Zea* in light of previous studies showing increasingly weak herbivore resistance from teosintes to highly-bred maize cultivars (Rosenthal and Dirzo 1997; Dávila-Flores et al. 2013). The consistent homogeneity in growth observed in both infested and non-infested seedlings suggest that the two maize types (landraces and inbred lines) and Balsas teosinte analyzed in this study are tolerant of feeding by the specialist phloem-feeder corn leafhopper at their seedling stage, and that a trade-off between tolerance and resistance is not evident among these plants. Other studies have similarly failed to find a negative correlation between herbivory tolerance and resistance. For instance, Mauricio et al. (1997) and Weinig et al. (2003) found no evidence of a negative genetic correlation between resistance and tolerance in *Arabidopsis thaliana*, and they hypothesized that stabilizing selection may be acting to maintain both defense strategies at intermediate levels. Moreover, other authors suggested that tolerance remains as a defensive trait in plants since it is not subjected to natural selection, unlike resistance, because tolerance might be promoted by stress factors other than herbivory, e.g., frosts and fires, and it is

unlikely that there is a selection pressure for an herbivore to overcome tolerance (Belsky et al. 1993; Rosenthal and Kotanen 1994). Mauricio et al. (1997) considered resistance and tolerance to be alternative strategies in the same plant, whereas Tiffin and Rausher (1999) found a positive correlation between the two defense strategies in *Ipomea* L.. My results suggested that selection mechanisms acting on tolerance and resistance in *Zea* are not correlated, given the increasing gradient in resistance (from teosintes to highly-bred maize) reported previously (Rosenthal and Dirzo 1997; Bellota et al. 2013; Davila-Flores et al. 2013).

My results suggested also that form and architecture in *Zea* are mediated by domestication and breeding; in the form analysis, one variable related to size (leaf length) in the first canonical axis clearly separated among Plant types, and even after controlling for size in the architecture analysis, the separation among Plant types continued to be significant. Morphological changes in *Zea* mediated by evolutionary transitions have been reported earlier. For example, Wang et al. (2005)showed that the differences between the cob from teosinte and the cob from maize resulted from a small mutation in a single gene. Moreover, while the Herbivory × Plant type interaction was not significant, the form analysis suggested a difference between infested and non-infested plants in teosinte per to the first canonical axis, suggesting differences in plant size as expected in a trade-off scenario.

A relevant aspect of this study concerns the feeding mode of corn leafhopper. Most studies on tolerance to date use vertebrates or chewing invertebrates that remove leaf tissue, bore in stems and/or remove meristematic tissues, and most plant fitness

parameters recorded measure plant ability to regrow after tissue removal or ability to relocate resources. In contrast, the injury caused by the corn leafhopper consists of removing phloem sap, and it is likely that plant responses to such injury might differ from responses triggered by tissue removal by chewing herbivores. A meta-analysis on tolerance of woody plants to plant sap-feeders suggested that plant responses to sap-feeder injury are likely to differ from responses triggered by herbivores with other feeding modes; for instance, while plant defenses against folivores were mostly based on secondary compounds, sap-feeders avoided these compounds by feeding on plant sap where their concentrations were low (Zvereva et al. 2010). Furthermore, the same meta-analysis suggested that defoliators increased the photosynthetic rate in woody plants (i.e. chlorophyll contents), whereas sap-feeders decreased it, so highlighting that plant responses to herbivory may depend on the type of feeding injury. The high degree of host specialization in corn leafhopper may have also played an important role in the tolerance responses displayed in maize and Balsas teosinte after feeding, as suggested earlier for sap-feeding herbivores (Zvereva et al. 2010), probably because specialist coevolution with hosts facilitates development of mechanisms to avoid detection by the plant. For example, Agrawal and Fishbein (2008) suggested that a macroevolutionary shift from resistance to tolerance in *Asclepias* spp. is the result of the dominance of specialist insects among the herbivore fauna feeding on that plant species. Moreover, tolerance responses have been reported to change with plant phenology (Briggs and Schultz 1990; Rosenthal and Kotanen 1994; Agrawal et al. 1999; Oriand et al. 2010; Zvereva et al. 2010). For instance, Orians et al. (2010) found that the cost of herbivory

defense is transient in willow seedlings (*Salix sericea* Marshall and *Salix eriocephala* Michx.), with fitness costs being evident only at early stages, and Briggs and Schultz (1990) showed that a trade-off between reproduction and tolerance is present in *Lotus corniculatus* only during seed filling. This suggests that it would be valuable to expand future experiments to include several phenological stages to confirm whether the tolerance seen here in maize and Balsas teosinte extends to growth stages beyond the seedling.

Planned, *a priori* contrasts revealed that shoot: root ratios were mediated by the domestication- but not the breeding transition, though independently from herbivory given that the Plant type × Herbivory interaction was not significant ($P = 0.670$ for dry weight, $P = 0.695$ for wet weight). The shoot: root ratios decreased with domestication, in the maizes compared to Balsas teosinte, as expected in a process of directed selection for higher productivity. Notably, in addition to the significant domestication effect on shoot: root ratios, root size and architecture seemed to differ, with numerous secondary roots and thinner primary roots in Balsas teosinte, and thicker and stronger primary roots and fewer secondary roots in the maizes (M.C.R. personal observation). Root hairs and secondary roots are primarily involved in absorption of minerals and water, whereas thicker roots are mainly for storage (van der Meijden et al. 1988), which may partially explain the lower shoot: root ratios in the maizes. However, future studies on tolerance to corn leafhopper in *Zea* should include both root and shoot morphology and biochemical analyses to elucidate other processes triggered by feeding.

Relocation of plant biomass (i.e. changes in shoot: root ratios) and metabolic resources (movement of secondary metabolites and/or their precursors) following herbivory are defensive strategies that are thought to be less costly than increases in metabolism to produce secondary compounds and/or promote regrowth to compensate for lost tissue; indeed, root: shoot ratios have been found to be good predictors of regrowth (Agrawal and Fishbein 2008). My results showed that herbivory by corn leafhopper did not effect changes in shoot: root ratios in Balsas teosinte or maize seedlings, suggesting that biomass allocation in seedlings is not affected by feeding injury by corn leafhoppers. Similarly, Zvereva et al. (2010) concluded that sap-feeders do not mediate biomass allocation in woody plants, hence tolerance responses triggered by herbivores in this feeding guild do not seem to be associated with resource allocation or growth pattern.

My study addressed changes in a limited set of seedling parameters, i.e., compensation, so it would be valuable for future studies to measure parameters related to photosynthesis rate to assess tolerance mechanisms other than compensation. Indirect effects on photosynthesis associated with herbivory include alterations of water transport, stomatal aperture, and sucrose transport and loading, as well as decreased stomatal conductance, and changes in nutrient status of leaves due to competition of insect-created sinks with plant sinks (Nabity et al. 2009; Zvereva et al. 2010). When no trade-off is detected between resistance and tolerance, plants may relocate defensive compounds to overcome insect damage without incurring fitness costs derived from regrowth. Gianoli and Niemeyer (1997) tested whether there was a negative correlation

between tolerance and resistance in wheat after aphid (Hemiptera) infestation, and found that a defensive compound, Hidroxamic acid, was relocated to aphid feeding sites rather than being synthesized *de novo*. Based on the resistance gradient found in *Zea* in earlier studies (Rosenthal and Dirzo 1997; Bellota et al. 2013; Davila-Flores et al. 2013) and the apparent absence of a trade-off with tolerance found in the present study, it is possible that a similar relocation process occurs in the suite of plant hosts I analyzed. In a preliminary plant hormone study carried out under the same experimental conditions described for this study, I found that the phytohormones salicylic acid (SA) and jasmonoyl-isoleucine (JA-Ile) were significantly higher in corn leafhopper-infested seedling compared to non-infested seedling across all Plant types ($P = 0.030$ and $P = 0.006$ respectively) after 7 days; SA levels seemed to increase from teosinte to inbred maize, while JA-Ile levels seemed to decrease, suggesting that teosinte and maize landrace responded to corn leafhopper herbivory by increasing JA-Ile level, while teosinte responded by increasing SA level (M.C.R., unpubl. data).

Overall, the results of my study were inconsistent with the predicted trade-off between resistance and tolerance, and suggested that Balsas teosinte and both maize landraces and inbred lines are tolerant to direct injury by corn leafhopper at the seedling stage; this may suggest that different selection forces act on tolerance and resistance (Belsky et al. 1993; Rosenthal and Kotanen 1994). My results are also inconsistent with predictions of the growth-differentiation hypothesis in that resource investment in differentiation (e.g., resistance) would trade-off with growth (i.e., tolerance) (Herms and Mattson 1992). In future studies, it would be worthwhile to assess potential effects of

corn leafhopper oviposition on tolerance, by including female corn leafhoppers, given that photosynthetic activity is affected in plants subjected to both feeding damage and oviposition compared to plants subjected only to feeding damage, and that oviposition is known to induce defense responses in plants (Hilker and Meiners 2011). Additionally, the results of my study also highlighted the necessity to broaden the scope of research of plant tolerance in *Zea* to include photosynthetic parameters (e.g., respiration, photosynthate' levels) among other fitness parameters, phenological stages beyond the seedling stage, and comprehensive phytohormone analysis to further elucidate the evolution of defense strategies in *Zea*.

CHAPTER III

WHAT A PEST LOOKS LIKE: TRADITIONAL AND GEOMETRIC MORPHOMETRICS DISCRIMINATE BETWEEN TWO SUBPOPULATIONS OF A MAIZE HERBIVORE ASSOCIATED TO DIFFERENT HOST PLANTS

Introduction

In a recent study, Medina et al. (2012) used AFLP markers to assess population structuring and genetic differentiation in corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae), a specialist herbivore on the genus *Zea* L. (Poaceae). Their results showed that corn leafhopper in Mexico is divided into at least two discrete subpopulations: one subpopulation associated with Perennial teosinte (*Z. diploperennis* Iltis, Doebly & Guzmán) and restricted to highland temperate forest habitat, and another subpopulation associated with maize (*Zea mays* ssp. *mays* L.) and its immediate ancestor, Balsas teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebly), with a widespread distribution in Mexico. They hypothesized that the evident genetic structuring was mediated by differences in overwintering dynamics among the subpopulations, host-associated differentiation (HAD), and anthropogenic activities. However, no morphological study was undertaken to examine whether genetic structuring and differentiation were correlated with morphological differentiation, if present. In another study using mtDNA haplotypes and AFLP markers, Bernal et al. (in prep.) conducted an analysis on corn leafhopper individuals from Mexico and Argentina, and confirmed the genetic structuring previously documented by Medina et al. (2012).

However, their results suggested that genetic structuring was lost in locations where maize and perennial teosinte coexisted, thus questioning the relevance of HAD in genetic differentiation of corn leafhopper populations. Bernal et al. (in prep.) suggested partial habitat isolation and immigrant inviability as probable mechanisms maintaining genetic differentiation between the two corn leafhopper subpopulations. They considered that the habitat of the corn leafhopper subpopulation on Perennial teosinte was partially isolated because maize (and Balsas teosinte) is largely absent from the plant's highland, temperate forest habitat. They hypothesized a role for immigrant inviability because prior studies suggested that corn leafhoppers of the maize subpopulation performed poorly when they developed on Perennial teosinte rather than maize in the laboratory (Dávila-Flores et al. 2013). Overall, Bernal et al. (in prep.) considered the corn leafhopper subpopulation on maize "pestiferous" because of its association with maize and its widespread distribution, while the subpopulation on Perennial teosinte was considered "wild" because of its association with a wild host and highly restricted distribution.

Morphometrics approaches have proven to be the most appropriate for describing and analyzing shape variation among species (Lawing and Polly 2010). In traditional morphometrics, variables are usually measured distances (i.e. lengths and widths) of structures, distances between landmarks, and angles and ratios, and it is not possible to recover the original form of the organism, whereas in geometric morphometrics a set of Cartesian coordinates are generated based on landmarks and semi-landmarks that describe the outline of the object, so capturing the geometry and variation of those

coordinates in all possible directions (Monteiro et al. 2002; Rohlf and Marcus 1993). Morphometrics analyses can also be coupled with molecular tools to facilitate the study of relationships between phenotype and genotype in the contexts of speciation, taxonomy or morphological differentiation between cryptic species (Adams and Funk 1997; Lawing and Polly 2010; Zinetti et al. 2013).

The goal of this study was to determine whether a correlation exists between genetic structuring previously found in corn leafhopper in western Mexico (Dávila-Flores 2012; Medina et al. 2012; Bernal et al. in prep.) and differentiation at the morphological level. Female and male individuals were collected from maize or Perennial teosinte in the presumed center of diversification for corn leafhopper and *Zea* in central, western Mexico, and traditional and geometric morphometrics approaches were applied respectively to body and wing measurements of the individuals. Form and shape were analyzed separately to discriminate changes in shape due to changes in size (i.e. allometry), and males and females were analyzed separately to consider known sexual dimorphism in corn leafhopper (Larsen and Nault 1994). The study's results are discussed in the contexts of host plants, environments, and anthropogenic influences may mediate insect morphology.

Methods

Specimen collection

The corn leafhopper specimens used in this study were collected from seven different locations in Colima and Jalisco states in western Mexico using sweep nets and aspirators. Specimens were collected from Perennial teosinte at three sites, Las Joyas, Corralitos, and San Miguel, and from maize at four sites, Tala, Talpitita, El Chante, and Caleras (Table 3.1). Maize is absent at the Las Joyas site, both maize and Perennial teosinte are present at the Corralitos and San Miguel sites, and maize is present and Perennial teosinte is absent at the remaining sites; at the Corralitos and San Miguel sites, specimens were collected from patches of Perennial teosinte that were distant at least 100 m from the nearest maize field. The Corralitos and San Miguel sites are hereafter referred to as "mixed-host sites."

Table 3.1 Collection sites, and their corresponding environmental and geographical coordinates, for corn leafhopper (*Dalbulus maidis*) specimens

Site	Distance from Las Joyas (Km)	Elevation (m.a.s.l.) [1]	Latitude	Longitude	Vegetation [2]
Las Joyas	--	1860	19°35'32.06"N	104°16'52.29"W	WTF
Corralitos	4	1810	19°36'54.62"N	104°18'21.88"W	WTF
San Miguel	13	1540	19°29'59.08"N	104°12'28.59"W	OPF
El Chante	15	912	19°42'27.64"N	104°12'14.20"W	A-STDF
Talpitita	55	370	19°42'47.37"N	104°47'18.33"W	A-STDF
Caleras	78	80	18°59'51.07"N	103°52'56.82"W	A-STHF
Tala	132	1327	20°39'5.29"N	103°42'26.85"W	A-STDF

[1] m.a.s.l.: Meters above sea level; [2]WTF = Wet, temperate forest, OPF = Oak-pine forest, A-STDF = Agricultural-Subtropical dry forest, A-STHF = Agricultural-Subtropical humid forest. Las Joyas: teosinte only site; San Miguel and Corralitos: teosinte and maize sites; El Chante, Talpitita, Caleras and Tala: maize only sites

Corn leafhoppers were collected using sweep-net and aspirator and immediately stored in 95% EtOH. Subsequently, they were dissected in the laboratory to take measurements of the head, thorax, wings, legs and reproductive organs. Dissected body parts were placed on microscope slides and a camera microscope (Dino Capture 2.0, New Taipei City, Taiwan) was used to take digital pictures and measurements (linear distances and areas) of the body parts.

Traditional morphometrics: body form and shape

The following measurements were taken: inter-antennal distance on the head; thorax width, height and length (each of these thoracic parameters is the average of three linear measurements, one taken in each of the three thoracic segments, pro-, meso- and metathorax); femur length and femur width (maximum linear distances were taken in every femur and then averaged per pair of legs); tibia length and tibia width (maximum linear distances were taken in every tibia and then averaged per pair of legs); forewing area (average surface area of the two forewings), hindwing area (average surface area of the two hindwings), and; maximum length of ovipositor and aedeagus in females and males, respectively. A total of 79 female individuals and 77 male individuals were used for the body analysis (Table 3.2).

Table 3.2 Sample sizes per collection site used for body and wing analyses of corn leafhopper (*Dalbulus maidis*) specimens

Site	Wings sample sizes				Body sample sizes	
	Female HW	Female FW	Male HW	Male FW	Females	Males
Las Joyas	14	19	7	7	11	6
Corralitos	13	20	12	16	11	12
San Miguel	10	16	10	15	9	9
El Chante	12	15	12	14	14	13
Talpitita	16	18	15	18	14	13
Caleras	9	15	13	15	14	14
Tala	0	5	7	13	6	10
TOTAL	**74**	**108**	**76**	**98**	**79**	**77**

HW = Hindwing, FW = Forewing

I performed a two-way MANOVA on shape and form (= size + shape) with host and collection site nested within host as factors to assess whether body morphologies of maize- and Perennial teosinte-associated corn leafhopper specimens varied among the two host plants, in correlation with genetic structuring and haplotype diversity previously found (Dávila-Flores 2012; Medina et al. 2012; Bernal et al. in prep.), followed by planned, *a priori* contrast comparisons (with Sidak's multiple comparisons correction) (Abdi and Williams 2010) to evaluate whether body morphologies of individuals from the same population varied across collecting sites. Prior to analyses all data were normalized by transforming to their natural logarithmic values, and then scaled by body size by dividing all variables by the corresponding thoracic volume (= geometric mean of ln-transformed thoracic measurements); *a priori* analyses showed that thoracic volume is a reliable predictor of body size (mass) in corn leafhopper;

(females: $R^2 = 0.81$, $P < 0.001$; males: $R^2 = 0.78$, $P < 0.001$) (data not shown). Data imputation using JPM Pro 11 was necessary for missing values: 5 aedeagus and 1 hind femur length among males (7.8 % of total data), and 1 thoracic height, 1 hind tibia width, and 1 fore tibia length among females (3.8 % of total data). Males and females were analyzed separately, and canonical centroid plots were generated for both body form and body shape for each sex. Correlations on canonical scores were used to determine the contribution of each independent variable (Pearson's r^2) to variation in the first two canonical axes of MANOVA centroid plots; only $R^2 > 0.50$ and $P < 0.05$ were considered in Results.

Additionally, I performed a two-way ANCOVA with host, and site nested within host as factors, and body size (= thoracic volume) as covariable to control for allometry (dependence of shape on size) to assess the magnitude of the differences in the means of body parts from the Perennial teosinte- and maize-associated populations. All linear measurement data were transformed to their natural logarithmic values to meet normality except for: fore femur width (FFW) and middle tibia width (MTW), where transformation was not needed; hind femur length (HFL), where squared root transformation was applied, and; fore tibia length (FTL), where any transformation proved successful for normality, hence transformation to ranks was chosen to run a non-parametric, two-way ANCOVA.

Both MANOVA and ANCOVA were performed in JMP Pro 10 (SAS Institute, Cary, North Carolina). Effect strength (n_p^2), which is the equivalent to R^2 for

multivariate analyses, was calculated in Microsoft Excel® using the **E** and **H** matrices from the output in JMP Pro 10 (Tabachnick and Fidell 2001).

Geometric morphometrics: wing form and shape

Digital photos of wings were made to acquire data for a geometric morphometrics analysis on wings from both maize- and Perennial teosinte-associated populations. A total of 206 forewings (98 from males and 108 from females) were included in the analysis (Table 3.2), and wing outline was defined by 5 landmarks and 52 semi-landmarks (Fig. 3.1a). In hindwings, 150 photos (76 from males and 74 from females) (Table 3.2) were analyzed, with 13 landmarks and 81semi-landmarks that defined wing outline (Fig. 3.1b). Both centroid and centroid size are important parameters for geometric morphometrics analyses, and were calculated for each wing; centroid is simply the center of the form, and centroid size is defined as the square root of summed squared distances from each landmark to the configuration centroid, and it is used in geometric morphometric approaches to transform all images to the same centroid size unit without changing the shape for posterior analysis (Monteiro et al. 2002; Zelditch et al. 2012). The software tpsDIG version 2.17 was used to digitize all landmarks and semi-landmarks in order to obtain its two-dimensional coordinates for posterior superimposition (i.e. translation, scaling, rotation) of all images.

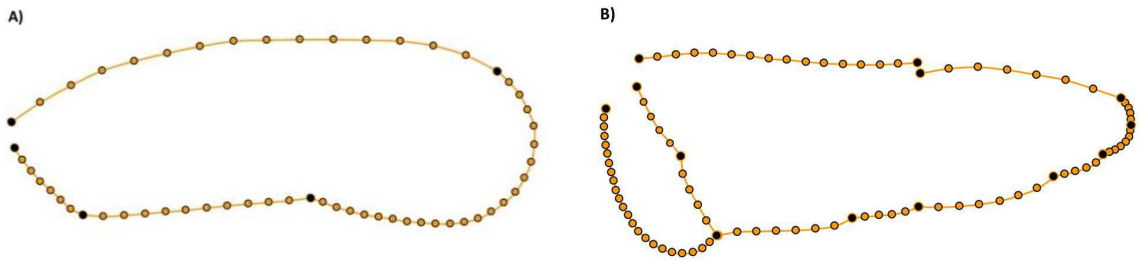


Figure 3.1 Landmark and semi-landmark placement in theforewing (A) and hindwing (B) of corn leafhopper (*Dalbulus maidis*) specimens. Landmarks are represented with black dots and semi-landmarks are represented with orange dots.

I performed a two-way MANOVA with host and site nested within host as independent variables for the form analysis, and a multifactorial MANOVA with host,

site nested within host and centroid size with all possible interactions as independent variables for the shape analysis in order to assess morphological differences between the wings of maize- and Perennial teosinte-associated corn leafhopper populations. MANOVAs were followed by planned, *a priori* contrast comparisons (with Sidak's multiple comparisons correction) (Abdi and Williams 2010) to determine whether the effects of host and site nested within host mediated wing form and shape. Analyses were performed for each wing (forewing, hindwing) and for each sex separately. In order to visualize differences in wings, if any, I generated thin-plate-spline transformations of landmark and semi-landmark positions for both forewing and hindwing with the software tpsRegr version 1.40 separately for males and females of Perennial teosinte- and maize-associated populations.As described previously, MANOVAs were carried out in JMP Pro 10, while effect strength (n_p^2) was calculated in Microsoft Excel® using the **E** and **H** matrices from the output in JMP (Tabachnick and Fidell 2001).

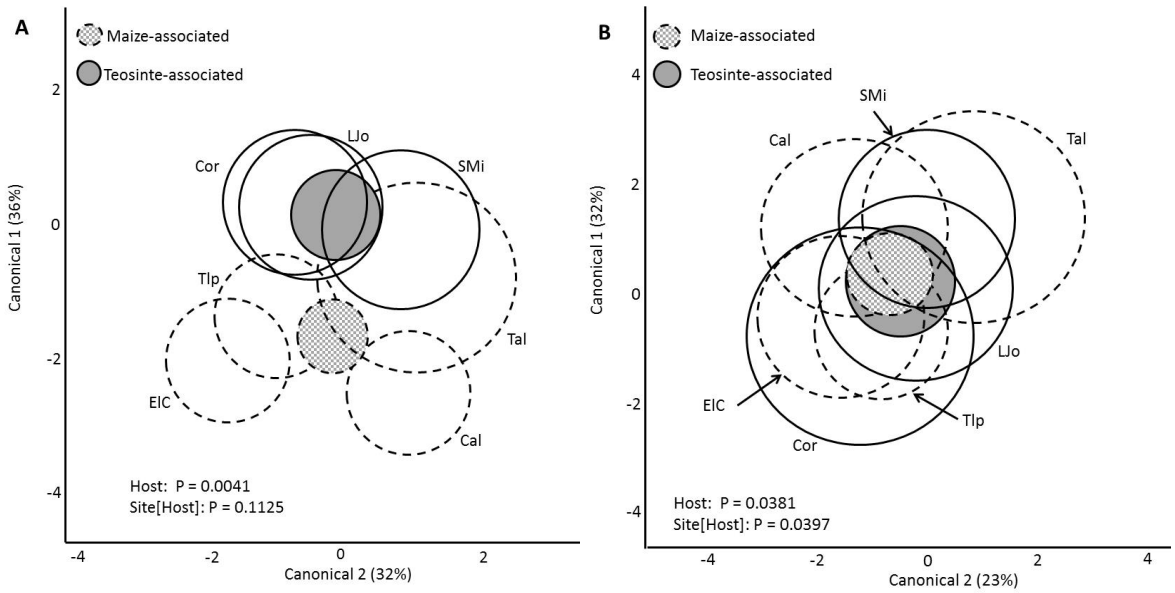
Results

Traditional morphometrics: body form and shape

Females

My results showed a significant and moderate effect of host ($P = 0.004$, $n_p^2 = 0.486$) and a non-significant effect of site nested within host ($P = 0.113$) on female body form (Fig. 3.2a) (Table 3.3). The vertical axis in the canonical plot for body form

explained 36% of variation, and discriminated between hosts. Correlations on canonical scores revealed that body size was the variable contributing the most to separation of both populations on that axis ($R^2 = 0.61$, $P < 0.001$). The horizontal axis on the body form canonical plot explained 32% of the variation, and discriminated among sites. For the body shape analysis on females, MANOVA revealed a significant and moderate effect of host ($P = 0.038$, $n_p^2 = 0.434$), as well as a significant, though moderate effect of site within host ($P = 0.040$, $n_p^2 = 0.352$) (Fig. 3.2b) (Table 3.3). Planned, *a priori* contrasts for the effect site within host (critical $P = 0.025$) on body shape showed no morphological differences between Perennial teosinte-associated individuals and mixed-host sites individuals ($P = 0.058$), and no morphological differences between individuals from mixed-host sites ($P = 0.041$) (Fig. 3.2c). The vertical axis of the canonical plot on body shape explained 32% of the variation, whereas the horizontal axis explained 23% of the variation (Fig. 3.2b).



C

Contrast	$F_{19, 47}$	P
Teosinte-associated vs. Mixed-host sites LJo vs. (SMi + Cor)	1.766	0.0578
Between Mixed-host sites SMi vs. Cor	1.8791	0.0405

Figure 3.2 Canonical centroid plots for body form and body shape in *Dalbulus maidis* females. Overall, MANOVA showed that Host (Perennial teosinte, maize) affected body form **(A)** and body shape **(B)**, and that the nested effect of Site within Host affected body shape only **(B)**. **A)** MANOVA on female body form showed significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.078$, $P = 0.005$, $F = 1.456$, $df = 120, 313.8$). **B)** MANOVA on female body shape showed significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.004$, $P < 0.0001$, $F = 1.485$, $df = 247, 540.5$). **C)** Planned contrasts for the nested effect of Site within Host (critical $P = 0.025$) on female body shape showing no morphological differences between Perennial teosinte-associated individuals and mixed-host sites individuals, and between mixed-host site individuals. Maize-associated type collecting sites are circled with dashed lines; Perennial teosinte-associated type collecting sites are circled with solid lines. Means of maize- and Perennial teosinte-associated individuals represented by filled circles. Cor: Corralitos, SMi: San Miguel, LJo: Las Joyas, Cal: Caleras, Tal: Tala, Tpl: Talpitita, ElC: El Chante.

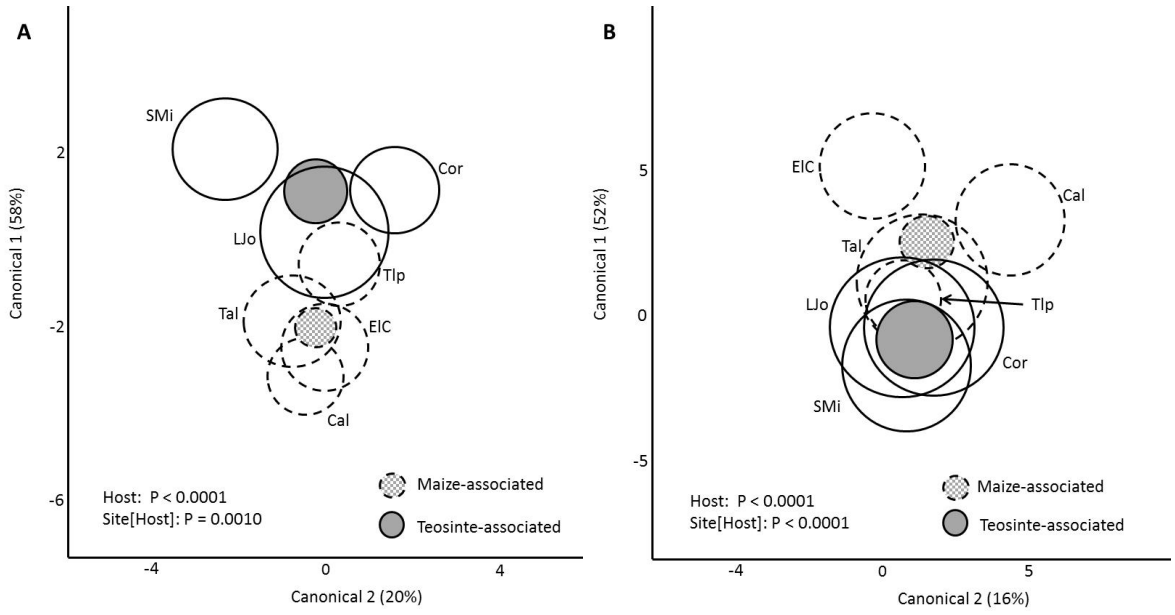
Table 3.3 MANOVA results for form and shape on body and wings of corn leafhopper (*Dalbulus maidis*) showing significance and strength for Host effect and Site within Host effect from morphometrics analyses

Variable	Females					Males				
	F-Test	**NumDF**	**DenDF**	**p-value**	**η_p^2**	**F-Test**	**NumDF**	**DenDF**	**p-value**	**η_p^2**
Body Form										
Host	2.5021	20	53	0.0041	0.486	6.1502	20	51	< 0.0001	0.707
Site[Host]	1.2153	100	263.23	0.1125	0.316	1.6436	100	253.47	0.0010	0.393
Body Shape										
Host	1.8988	19	47	0.0381	0.434	3.8344	19	45	< 0.0001	0.618
Site[Host]	1.3339	95	233.29	0.0397	0.352	1.9576	95	223.56	< 0.0001	0.454
Forewing Form										
Host	4.5121	8	94	< 0.0001	0.277	7.4455	7	85	< 0.0001	0.380
Site[Host]	2.3931	40	412.53	< 0.0001	0.188	2.5967	35	359.99	< 0.0001	0.202
Forewing Shape										
Host	1.5266	7	88	0.1687	0.108	2.1235	6	79	0.0597	0.138
Site[Host]	1.3730	35	372.61	0.0821	0.114	2.1951	30	318	0.0005	0.172
Hindwing Form										
Host	3.1257	17	52	0.0008	0.505	5.9449	17	53	< 0.0001	0.656
Site[Host]	1.9047	68	206.38	0.0003	0.386	1.9000	85	260.63	< 0.0001	0.383
Hindwing Shape										
Host	1.1908	16	47	0.3099	0.288	1.4088	16	47	0.1789	0.324
Site[Host]	1.0191	64	186.27	0.4500	0.259	1.4538	80	230.58	0.0169	0.335

η_p^2 = effect strength. Site[Host] = nested effect of site within host. Weak effect $\eta_p^2 < 0.2$; Moderate effect $0.2 > \eta_p^2 < 0.5$; Strong effect $\eta_p^2 > 0.5$

Males

MANOVA on body form and body shape for males yielded similar results to those in females. For body form, the host effect was significant and strong ($P < 0.0001$; $n_p^2 = 0.707$) and the effect of site within host was also significant, but moderate ($P = 0.001$; $n_p^2 = 0.393$) (Fig. 3.3a) (Table 3.3). Planned, *a priori* contrasts revealed no morphological differentiation between Perennial teosinte-associated individuals and mixed-host site individuals ($P = 0.269$), but showed morphological differences between individuals from mixed-host sites, San Miguel and Corralitos ($P < 0.001$) (Fig. 3.3c). The vertical axis in the body form analysis explained 58% of the variation, and discriminated between hosts. The variable with the major contribution on that axis was body size ($R^2 = 0.72$, $P < 0.001$). The horizontal axis in the body form plot explained 20% of the variation (Fig. 3.3a). In the body shape analysis, the effect of host was significant and strong ($P < 0.0001$; $n_p^2 = 0.618$), and the nested effect of site within host was also significant, but moderate ($P < 0.0001$; $n_p^2 = 0.454$) (Fig. 3.3b) (Table 3.3). Planned contrasts for the nested effect in body shape revealed no morphological differentiation between Perennial teosinte-associated and mixed-host sites individuals ($P = 0.995$), and between mixed-host sites individuals ($P = 0.029$) (Fig. 3.3d). The vertical axis in the body shape analysis explained 52% of total variation and discriminated between hosts while the horizontal axis explained 16% of the variation (Fig. 3.3b).



C

Contrast	$F_{20, 51}$	P
Teosinte-associated vs. Mixed-host sites LJo vs. (Smi + Cor)	1.231	0.2692
Between Mixed-host sites SMi vs. Cor	3.1097	0.0006

D

Contrast	$F_{19, 45}$	P
Teosinte-associated vs. Mixed-host sites LJo vs. (SMi + Cor)	0.326	0.9949
Between Mixed-host sites SMi vs. Cor	1.9955	0.0292

Figure 3.3 Canonical centroid plot for body form and body shape in *Dalbulus maidis* males. Overall, MANOVA showed that Host (Perennial teosinte, maize) and the nested effect Site within Host affected both body form **(A)** and body shape **(B)**. **A)** MANOVA on male body form showing significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.032$, $P < 0.0001$, $F = 2.05$, df = 120, 302.2). B) MANOVA on male body shape showing significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.007$, $P = 0.035$, $F = 1.215$, df = 247, 518.9). **C)** Planned contrasts for the nested effect of Site within Host on male body form showing significant differences betweenmixed-host site individuals and no significant differences between Perennial teosinte-associated individuals and mixed-host site individuals. **D)** Planned contrasts for the effect Site within Host on male body shape showing no significant differences between Perennial teosinte-associated individuals and mixed-host site individuals, and showing no significant differences between mixed-host site individuals. Maize-associated type collecting sites are circled with dashed lines; Perennial teosinte-associated type collecting sites are circled with solid lines. Means of Perennial teosinte- and maize-associated individuals represented by filled circles. Cor: Corralitos, SMi: San Miguel, LJo: Las Joyas, Cal: Caleras, Tal: Tala, Tlp: Talpitita, ElC: El Chante.

Size differences in individual body parts

ANCOVA confirmed significant differences for several body parts between maize- and Perennial teosinte-associated individuals, for both males and females (Fig.3.4) (Table 3.4). In the case of females, maize-associated specimens showed body parts with comparatively smaller dimensions than Perennial teosinte-associated specimens in 7 of 19 variables [hind femur width (HFW), hindwing (Hind), reproductive organ (REP), middle femur width (MFW), thorax width (TW), hind femur length (HFL), fore femur width (FFW)], and a part with larger dimensions [thorax length (TL)]. Similarly, males associated with maize had body parts with smaller dimensions relative to those associated with Perennial teosinte in 13 of 19 variables [hind femur width (HFW), hindwing (Hind), middle femur width (MFW), fore tibia width (FTW), hind femur length (HFL), hind tibia length (HTL), forewing (Fore), middle femur length (MFL), hind tibia width (HTW), thorax width (TW), middle tibia length (MTL), fore tibia length (FTL), fore femur width (FFW)], and two parts with larger dimensions [reproductive organ (REP), thorax length (TL)] (Fig. 3.4) (Table 3.4).

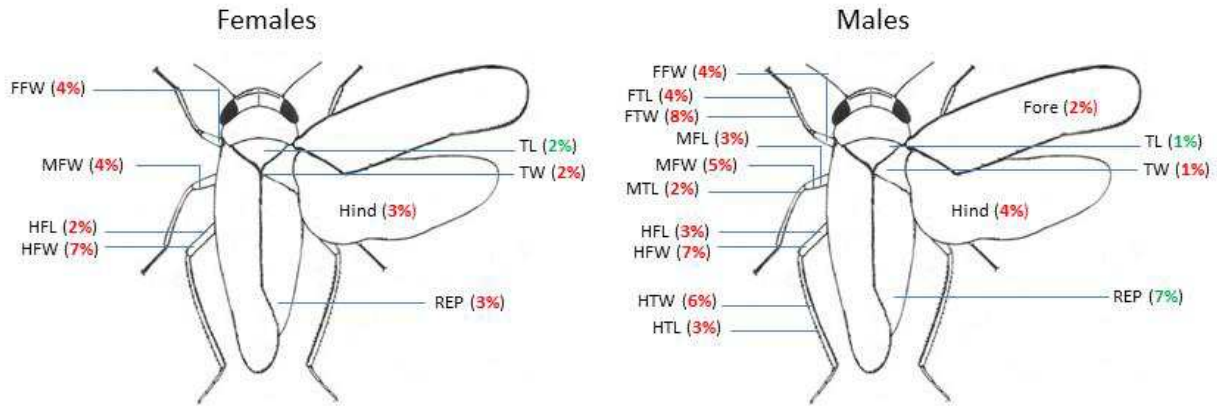


Figure 3.4 Body shape differences of maize-associated corn leafhoppers (shown) relative to Perennial teosinte-associated corn leafhoppers (not shown). Overall, ANCOVA indicated that body parts in maize-associated individuals had smaller or similar dimensions than in Perennial teosinte-associated ones, and a higher number of body variables were smaller in males compared to females. Values in red indicate decreases in size and values in green indicate increase in size. Fore femur width (FFW), Fore tibia width (FTW), Hind femur length (HFL), Hind femur width (HFW), Hind tibia length (HTL), Middle femur length (MFL), Middle femur width (MFW), Middle tibia length (MTL), Reproductive organ length (REP), Thorax Length (TL), Thorax Width (TW), Fore wing area (Fore), Hind wing area (Hind). (Females: 79 individuals; Males: 77 individuals) (Drawing modified from Wilson & Turner 2010).

Table 3.4 Body shape variable means from ANCOVA on maize-associated and teosinte-associated corn leafhopper populations showing significant changes in 8 variables for females and 15 variables for males

Females					Males				
Variable	**Maize**	**Teosinte**	**F**	**P**	**Maize**	**Teosinte**	**F**	**P**	**Variable**
HFW	0.121	0.130	16.199	**<.001**	0.120	0.130	35.344	**<.001**	**HFW**
Hind	1.478	1.521	10.677	**0.002**	1.416	1.474	26.054	**<.001**	**Hind**
REP	0.409	0.421	9.227	**0.003**	0.138	0.146	21.781	**<.001**	**MFW**
MFW	0.142	0.149	8.563	**0.005**	0.072	0.078	19.420	**<.001**	**FTW**
TW	0.879	0.892	7.454	**0.008**	0.341	0.318	13.990	**<.001**	**REP**
TL	1.536	1.510	7.403	**0.008**	0.879	0.905	10.556	**0.002**	**HFL**
HFL	0.910	0.927	6.948	**0.010**	1.669	1.716	10.201	**0.002**	**HTL**
FFW	0.130	0.135	5.029	**0.028**	1.392	1.423	8.579	**0.005**	**Fore**
HTW	0.111	0.115	3.747	0.057	0.584	0.599	7.754	**0.007**	**MFL**
FTW	0.070	0.073	3.559	0.063	0.111	0.119	7.408	**0.008**	**HTW**
MFL	0.597	0.607	3.288	0.074	0.831	0.843	6.789	**0.011**	**TW**
MTW	0.073	0.075	2.402	0.126	0.773	0.788	6.458	**0.013**	**MTL**
Fore	1.471	1.487	1.570	0.214	0.658	0.689	6.121	**0.016**	**FTL**
HTL	1.778	1.797	1.090	0.300	0.129	0.133	5.050	**0.028**	**FFW**
MTL	0.779	0.788	0.965	0.329	1.455	1.435	4.857	**0.031**	**TL**
IAD	0.350	0.353	0.591	0.445	0.074	0.076	2.231	0.140	MTW
FTL	0.661	0.680	0.527	0.470	0.333	0.331	0.206	0.652	IAD
FFL	0.645	0.640	0.378	0.541	0.633	0.633	0.005	0.946	FFL
TH	0.819	0.821	0.113	0.738	0.782	0.782	0.001	0.971	TH

Inter-antennal distance (IAD), Fore femur length (FFL), Fore femur width (FFW), Fore tibia length (FTL), Fore tibia width (FTW), Hind femur length (HFL), Hind femur width (HFW), Hind tibia length (HTL), Hind tibia width (HTW), Middle femur length (MFL), Middle femur width (MFW), Middle tibia length (MTL), Middle tibia width (MTW), Reproductive organ length (REP), Thorax Height (TH), Thorax Length (TL), Thorax Width (TW), Fore wing area (Fore), Hind wing area (Hind). *Non-parametric ANOVA. Boldface indicates significant changes in variables.

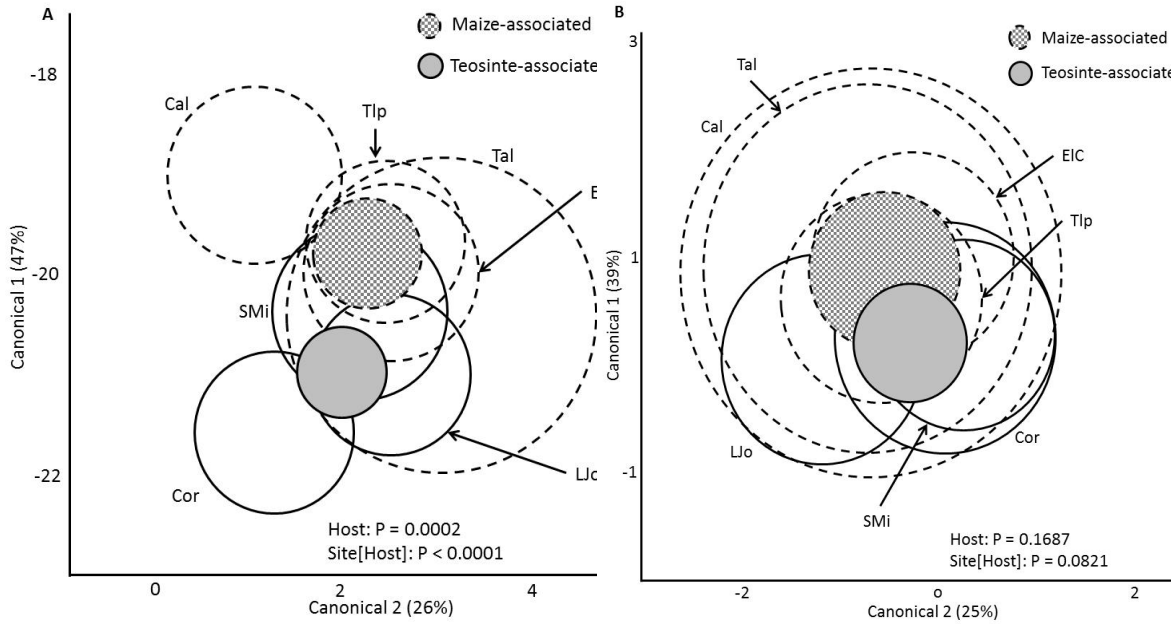
Geometric morphometrics: wing form and shape

Forewing in females

MANOVA on female forewing form showed significant but weak effects of host ($P < 0.001$; $n_p^2 = 0.277$) and site within host ($P < 0.0001$; $n_p^2 = 0.188$) (Fig. 3.5a) (Table 3.3). Planned, *a priori* contrasts showed significant differences between Perennial teosinte-associated and mixed-host sites specimens ($P < 0.001$), and between specimens from mixed-host sites ($P = 0.007$) (Fig. 3.5c). MANOVA on female forewing shape showed no significant effects of host ($P = 0.169$), and no significant effect of site within host ($P = 0.082$) (Fig. 3.5b).

Forewing in males

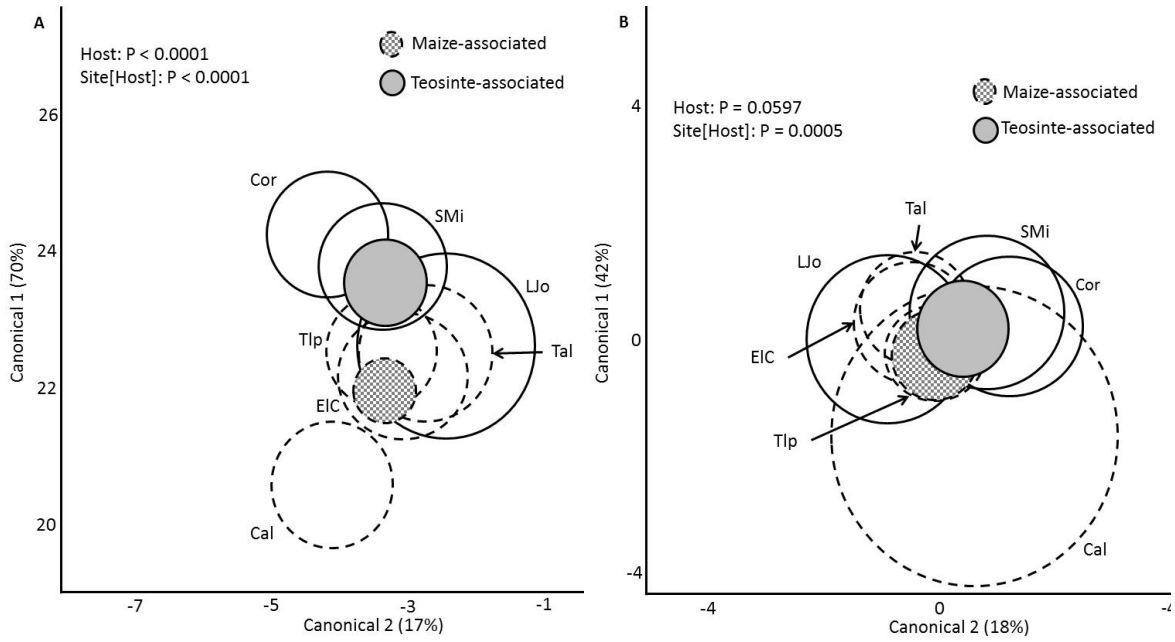
MANOVA on male forewing form showed a significant but moderate effect of host ($P < 0.0001$; $n_p^2 = 0.380$) and a significant but weak effect of site within host ($P < 0.0001$; $n_p^2 = 0.202$) (Fig. 3.6a) (Table 3.3). Planned, *a priori* contrasts revealed form differences between Perennial teosinte-associated and mixed-host sites specimens ($P = 0.003$) but not between specimens from mixed-host sites ($P = 0.037$) (Fig. 3.6c). MANOVA on male forewing shape showed no significant effect of host ($P = 0.060$), and a significant but weak effect of site within host ($P < 0.001$; $n_p^2 = 0.172$) (Fig. 3.6b) (Table 3.3). Planned, *a priori* contrasts showed differences in shape between Perennial teosinte-associated and mixed-host sites specimens ($P = 0.006$) but not between mixed-host sites specimens ($P = 0.047$) (Fig. 3.6d).



C

Contrast	$F_{8, 94}$	P
Teosinte-associated vs. Mixed-host site LJo vs. (Cor + SMi)	3.7221	0.0008
Between Mixed-host site Cor vs. SMi	2.8821	0.0065

Figure 3.5 Canonical centroid plot for forewing form and shape in *Dalbulus maidis* females. Overall, MANOVA showed that Host (Perennial teosinte, maize) and Site within Host affected forewing form only**(A)**. **A)** MANOVA on forewing form showing significant difference between maize- and Perennialteosinte-associated individuals (Wilks' $\lambda = 0.282$, $P < 0.0001$, $F = 2.848$, df = 48, 466.6). **B)** MANOVA on forewing shape showing no significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.168$, $P < 0.0001$, $F = 2.025$, df = 91, 557.1). **C)** Planned contrasts for the nested effect Site within Host on forewing form showing significant differences between Perennial teosinte-associated and mixed-host site individuals, and between mixed-host site individuals. Maize-associated type collecting sites are circled with dashed lines; Perennial teosinte-associated type collecting sites are circled with solid lines. Means of maize- and Perennial teosinte-associated individuals represented by filled circles. Cor: Corralitos, SMi: San Miguel, LJo: Las Joyas, Cal: Caleras, Tal: Tala, Tlp: Talpitita, ElC: El Chante.



C

Contrast	$F_{7, 85}$	P
Teosinte-associated vs. "Mixed" LJo vs. (Cor + SMi)	3.4035	0.0030
Between "Mixed" Cor vs. SMi	2.2547	0.0374

D

Contrast	$F_{6, 79}$	P
Teosinte-associated vs. "Mixed" LJo vs. (Cor + SMi)	3.3207	0.0057
Between "Mixed" Cor vs. SMi	2.2513	0.0467

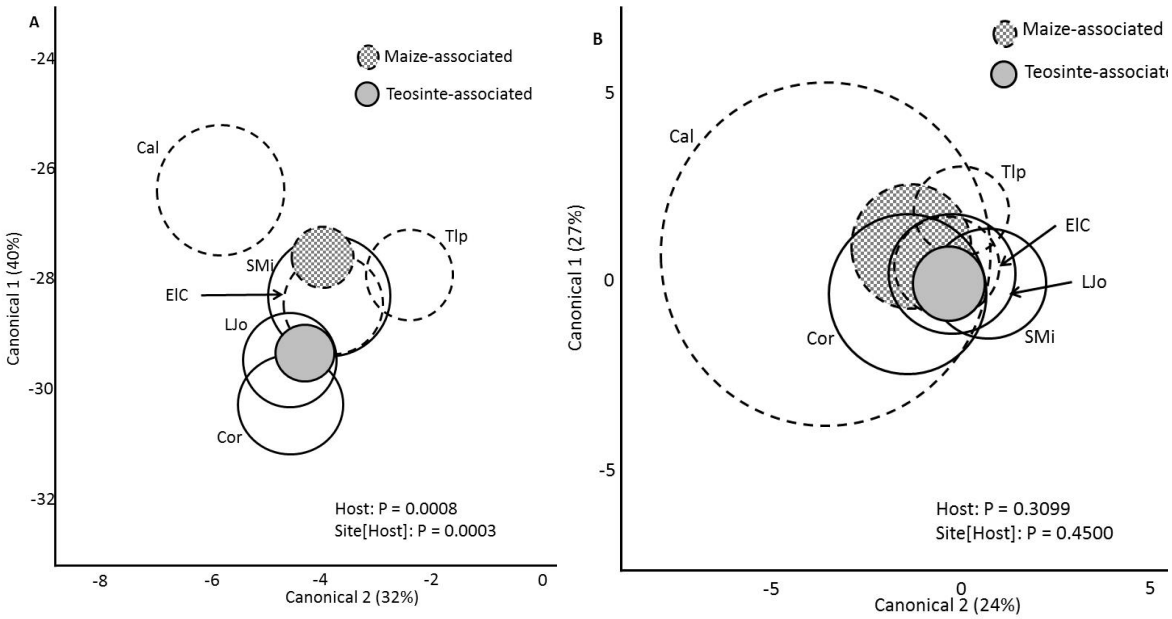
Figure 3.6 Canonical centroid plot for forewing form and shape in *Dalbulus maidis* males. Overall, MANOVA showed that Host (Perennial teosinte, maize) affected forewing form but not shape **(A)**, and that Site within Host affected both forewing form **(A)** and shape **(B)**. **A)** MANOVA on forewing form showing significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.246$, $P < 0.0001$, $F = 3.340$, $df = 42, 402.1$). **B)** MANOVA on forewing shape showing no significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.171$, $P < 0.0001$, $F = 2.136$, $df = 78, 441.7$). **C)** Planned contrasts for the effect Site within Host showing significant differences in forewing form between Perennial teosinte-associated individuals and mixed-host site individuals, but not between mixed-host sites individuals; D) Planned contrasts for the effect Site within Host showing significant differences in forewing shape between Perennial teosinte-associated individuals and mixed-host sites individuals, but not between mixed-host site individuals. Maize-associated type collecting sites are circled with dashed lines; Perennial teosinte-associated type collecting sites are circled with solid lines. Means of maize- and teosinte-associated individuals represented by filled circles. Cor: Corralitos, SMi: San Miguel, LJo: Las Joyas, Cal: Caleras, Tal: Tala, Tlp: Talpitita, ElC: El Chante.

Hindwing in females

MANOVA on female hindwing form showed a significant and strong effect of host ($P < 0.001$; $n_p^2 = 0.505$), and a significant, but moderate effect of site within host ($P < 0.001$; $n_p^2 = 0.386$) (Fig. 3.7a) (Table 3.3). Planned, *a priori* contrasts revealed no significant differences in form between Perennial teosinte-associated individuals and mixed-host sites individuals ($P = 0.490$), and a significant difference between mixed-host sites specimens ($P = 0.012$) (Fig. 3.7c). MANOVA on female hindwing shape showed no effect of host ($P = 0.310$) or site within host ($P = 0.450$) (Fig. 3.7b).

Hindwing in males

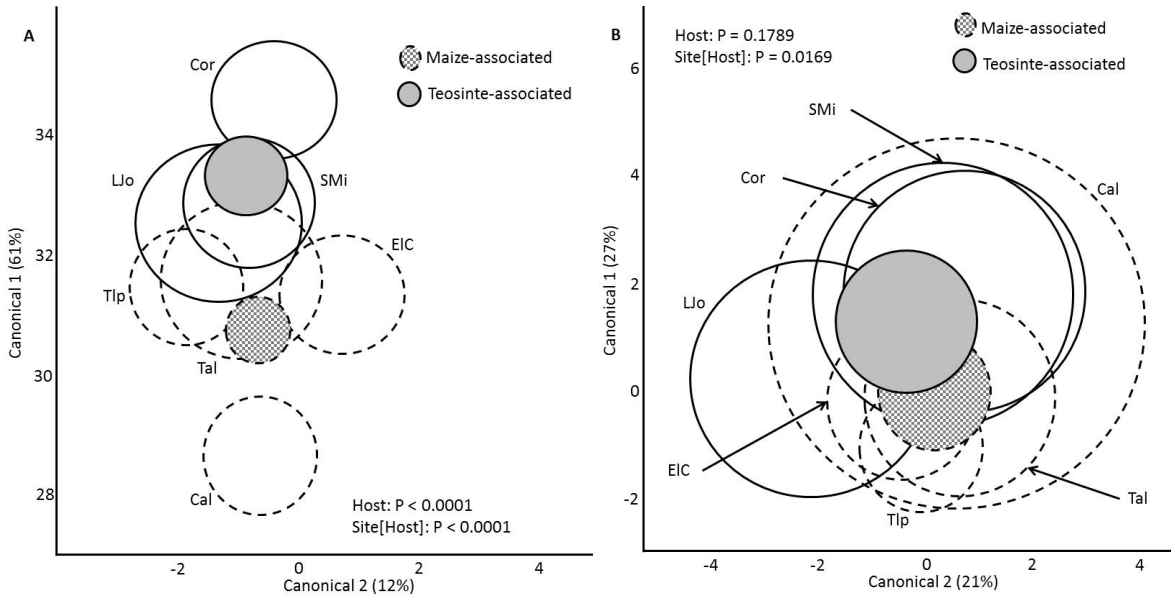
MANOVA on male hindwing form showed a significant and strong effect of host ($P < 0.0001$; $n_p^2 = 0.656$), and a significant but, moderate effect of site within host ($P < 0.0001$; $n_p^2 = 0.383$) (Fig. 3.8a) (Table 3.3). Planned, *a priori* contrasts showed no significant differences between Perennial teosinte-associated individuals and mixed-host sites individuals ($P = 0.224$), and between mixed-host site individuals ($P = 0.151$) (Fig. 3.8c). MANOVA on male hindwing shape showed no effect of host ($P = 0.179$), and a significant but moderate effect of site within host ($P = 0.017$; $n_p^2 = 0.335$) (Fig. 3.8b) (Table 3.3). Planned, *a priori* contrasts showed significant differences between Perennial teosinte-associated individuals and mixed-host site individuals ($P = 0.004$), but not between mixed-host sites specimens ($P = 0.854$) (Fig. 3.8d).



C

Contrast	$F_{17, 52}$	P
Teosinte-associated vs. Mixed-host site LJo vs. (Cor + SMi)	0.9833	0.4901
Between Mixed-host site Cor vs. SMi	2.2799	0.0118

Figure 3.7 Canonical centroid plot for hindwing form and shape in *Dalbulus maidis* females. Overall, MANOVA showed that Host (Perennial teosinte, maize) and Site within Host affected body form only **(A)**. **A)** MANOVA on hindwing form showing significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.0763$, $P < 0.0001$, $F = 2.115$, df = 85, 255.8). **B)** MANOVA on female hindwing shape showing no significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.016$, $P = 0.0007$, $F = 1.477$, df = 176, 451.4. **C)** Planned contrast for the effect Site within Host on hindwing form showing no significant difference between Perennial teosinte-associated and mixed-host sites individuals, and a significant difference between mixed-host sites individuals. Maize-associated type collecting sites are circled with dashed lines; Perennial teosinte-associated type collecting sites are circled with solid lines. Means of maize- and Perennial teosinte-associated indoviduals represented by filled circles. Cor: Corralitos, SMi: San Miguel, LJo: Las Joyas, Cal: Caleras, Tlp: Talpitita, ElC: El Chante.



C

Contrast	$F_{17, 53}$	P
Teosinte-associated vs. Mixed-host site LJo vs. (SMi + Cor)	1.3080	0.2240
Between Mixed host sites SMi vs. Cor	1.44889	0.1517

D

Contrast	$F_{16, 47}$	P
Teosinte-associated vs. Mixed-host site LJo vs. (SMi + Cor)	2.6733	0.0045
Between Mixed-host site SMi vs. Cor	0.6166	0.8543

Figure 3.8 Canonical centroid plot for hindwing form and shape in *Dalbulus maidis* males. Overall, MANOVA showed that Host (Perennial teosinte, maize) affected hindwing form only **(A)**, and that Site within Host affected both hindwing form **(A)** and shape **(B)**. **A)** MANOVA on hindwing form showing significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.039$, $P < 0.0001$, $F = 2.335$, df = 102, 309.2). **B)** MANOVA on hindwing shape showing no significant difference between maize- and Perennial teosinte-associated individuals (Wilks' $\lambda = 0.004$, $P < 0.0001$, $F = 1.717$, df = 208, 505.9). **C)** Planned contrasts for the effect Site within Host on hindwing form showing no significant differences between Perennial teosinte-associated individuals and mixed-host site individuals, nor between individuals from mixed-host sites. **D)** Planned contrasts for the effect Site within Host on hindwing shape showing a significant difference between Perennial teosinte-associated individuals and mixed-host site individuals, but not between mixed-host sites individuals. Maize-associated type collecting sites are circled with dashed lines; Perennial teosinte-associated type collecting sites are circled with solid lines. Means of maize- and Perennial teosinte-associated individuals represented by filled circles. Cor: Corralitos, SMi: San Miguel, LJo: Las Joyas, Cal: Caleras, Tal: Tala, Tlp: Talpitita, ElC: El Chante.

Form variation in **Dalbulus maidis** *wings*

I generated thin-plate-spline transformation grids to visualize differences in shape, if any, in both wings for each sex and subpopulation separately. Differences between populations due to host plant were non-significant in the shape analysis, thus I only generated thin-plate-spline transformations to visualize differences in wings due to form (= size + shape). Differences in forewings were more evident in the posterior margins and distal portions; the anal area in Perennial teosinte-associated individuals is more angled and broader, whereas in maize-associated individuals the same area is more rounded and narrower. Also, the distal half of the wing in Perennial teosinte-associated individuals is narrower and the outer margin is in straight alignment relative to the basal portion, while in maize-associated individuals the distal portion is more expanded near the outer margin and slightly directed downwards. In the hindwings, different sections showed differences between individuals from the different host plants: in the basal portion of the wing in Perennial teosinte-associated individuals the anal area is broader and larger and the attachment to the body is broader as well, while in maize-associated individuals the anal area is smaller and the attachment to the body is narrower; the distal portion of the wing is narrower with a pointed outer margin in individuals associated to Perennial teosinte, while in maize-associated individuals the outer margin is broader and rounded (Fig. 3.9). Additionally, I generated wing images showing the variation in the position and direction of the landmark and semi-landmark vectors for detailed visualization of morphological differentiation between Perennial teosinte-associated and

maize-associated individuals relative to the average wing shape for both populations (Fig. 3.10).

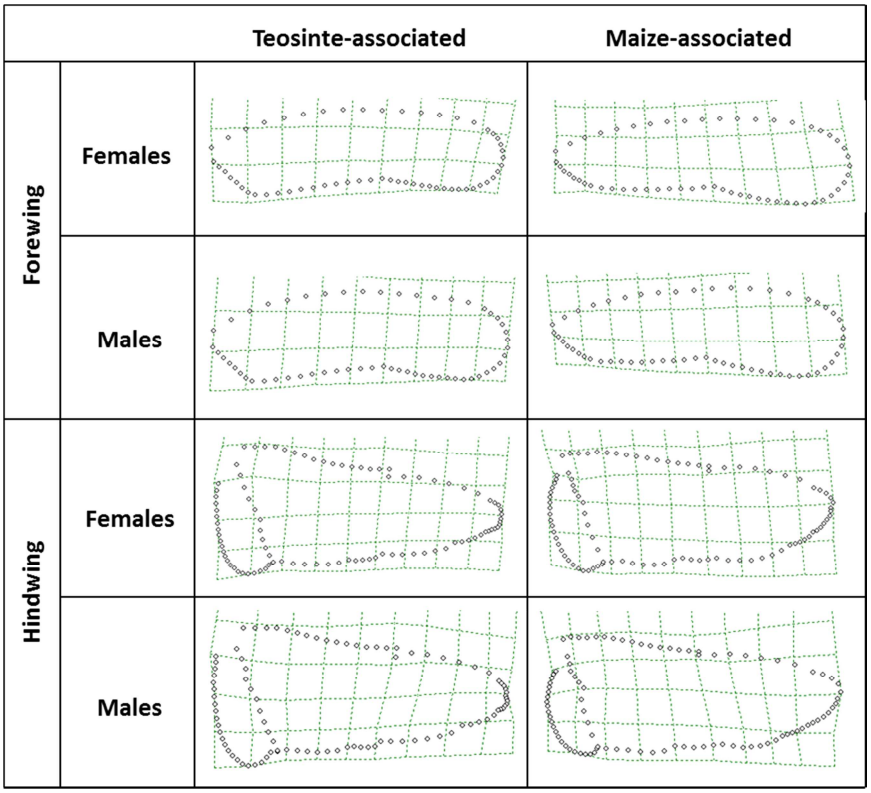


Figure 3.9 Thin-plate-spline transformations on form (size + shape) for both forewings and hindwings of maize-associated and teosinte-associated corn leafhopper specimens (Augmented 10×). Overall, differences were more subtle in forewings compared to hindwings, with a more expanded anal area (posterior margin) and narrower distal margin in Perennial teosinte-associated individuals compared to maize-associated individuals; differences in hindwings were more evident, with an enlarged anal area, a more pointed distal margin and a broader attachment to the body in Perennial teosinte compared to maize associated individuals. Differences in shape were due to allometry.

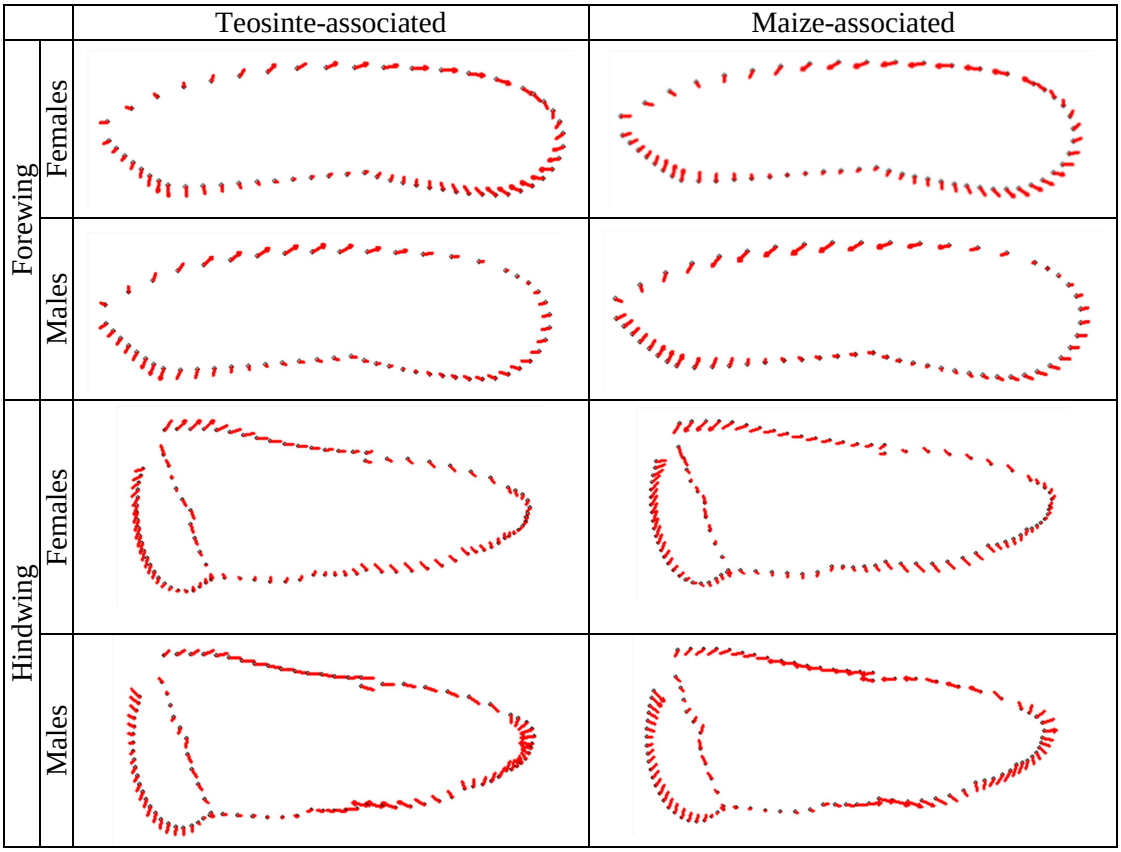


Figure 3.10 Landmark and semi-landmark vectors in forewings and hindwings of corn leafhopper (*Dalbulus maidis*) specimens. Arrows indicate directions and the length of vectors indicates the variation in shape of the wing relative to the average wing shape for both populations.

Discussion

The results of the present study confirmed morphological divergence at body and wing levels between maize-associated and Perennial teosinte-associated corn leafhopper individuals from western Mexico. This morphological differentiation is consistent with genetic structuring previously documented for corn leafhopper inhabiting the same region in Mexico (Dávila-Flores 2012; Medina et al. 2012; Bernal et al. in prep.). Traditional morphometrics on body form showed that Perennial teosinte-associated specimens are generally larger than those associated to maize, and males showed more variation in body shape than females. The effect strength, estimate of partial variance explained (n_p^2), for host plant was strong for males and moderate for females, whereas it was moderate for the both sexes for Site within Host. Differences in body shape between populations were evident, even after controlling for allometry. In contrast, geometric morphometrics analysis on wing form and shape revealed that differences between Perennial teosinte- and maize-associated populations are present, but are highly dependent on allometry. The effect strength (n_p^2) of host plant was stronger for hindwings and weaker for forewings in both sexes, and for the nested effect of site within host it varied from weak to moderate across sexes and type of wing.

The number of variables showing size changes in both female and male corn leafhoppers is consistent with the host effect strengths for each sex: the effect of hosts in males was stronger, thus a higher number of shape variables were influenced, whereas the effect of hosts was moderate in females, and consequently fewer variables were affected. It is likely that mechanisms controlling changes in body shape and size for

females are more constrained than in males given that those traits may be related to slower development rates in females, and a relationship in females between body size and weight and their ability to nurture and mature eggs (de Oliveira et al. 2004; Larsen and Nault 1994). Moreover, it was notable that reproductive organ length in males was one of the few variables that increased in maize-associated specimens, along with thorax length, suggesting that reproductive success is independent from changes in other body parts, and selective pressure might be directed towards preservation of a genitalia size that enhances the likelihood of mating with females associated with the same host plant. In a recent study, Horton et al. (2008) showed that *Anthocoris* sp. (Hemiptera: Anthocoridae) males with a longer aedeagus were more successful inseminating females from the same geographic population than males from different populations with smaller genitalia. Interestingly, not only was an important host effect on body form and shape evident in my results, but a geographic/habitat effect was evident as well, as indicated by the moderate nested effect in the shape analysis (i.e., after removing size from variables). Furthermore, in the case of males the nested effect became stronger after controlling for allometry, thus suggesting that environmental conditions in the collecting sites have a relevant influence in the morphological divergence I found. This is consistent with other studies that found that environmental factors such as temperature, photoperiod, food quality and availability, density of conspecifics, relative humidity, and rainfall were associated with differentiation of corn leafhopper (de Oliveira et al. 2004; Larsen and Nault 1994).

Contrasts among collecting sites revealed that morphological differentiation was maintained between mixed-host collecting sites, where both Perennial teosinte and maize grow in close proximity, relative to the Perennial teosinte-only site, Las Joyas. This result contrasts with that of Bernal et al. (in prep.) who found that genetic structuring between those sites was lost. Thus, my results suggested that differentiation at the morphological level is independent of genetic differentiation and controlled by a different mechanism, and is maintained regardless of the mixed-host condition of the collecting sites. In other studies, phenotypic plasticity has been previously reported in corn leafhopper as a mechanism for morphological adaptation to different environments, and the influence of plant host in insect morphological traits has been extensively documented in other insects (Barman 2011; de Oliveira et al. 2004; Gillham and Claridge 1994; Harrison 1980; Jorge et al. 2011; Larsen and Nault 1994). Moreover, one of the mixed-host sites (Corralitos) is 4 km distant from the Perennial teosinte-only site (Las Joyas), while the other mixed-host site (San Miguel) is 13 km distant so that it is probable that movement of teosinte-associated individuals occurs at a higher rate between the first site and the Perennial teosinte-only site. This seems to be supported by the degree of overlap between Las Joyas and Corralitos specimens in the canonical centroid plots compared to the overlap between Las Joyas and San Miguel specimens. Importantly, body size appears to be the variable separating the mixed-host site San Miguel from Las Joyas and mixed-host site Corralitos because the three collecting sites showed broader overlapping after controlling for allometry.

Overall, body size was expected to be larger in maize-associated specimens, as seen in other insects feeding on host plants with relatively high nutritional quality, such as cultivated maize (Nevo and Coll 2001). For example, Dávila-Flores et al. (2013) showed that adult size (mass) of putative maize associated corn leafhoppers was smaller when forced to develop on Perennial teosinte. However, Perennial teosinte-associated specimens were larger, thus suggesting that factors other than host quality are influencing body size, as well as shape. In a recent study, de Oliveira et al. (2004) reported elevation a relevant factor for body size and weight in corn leafhopper in Brazil, and showed a positive correlation between elevation and those traits. Those results are consistent with my findings in that Perennial teosinte-associated specimens were collected > 1500 m.a.s.l. (meters above sea level), whereas maize-associated specimens were collected < 1350 m.a.s.l. However, a more detailed analysis that integrates elevation is needed for confirmation. Additionally, it is probable that other factors, such as habitat composition and availability of overwintering feeding-hosts might influence corn leafhopper size as well; Perennial teosinte sites are located in a wet temperate forest habitat with a high diversity of plants and year round availability of alternative feeding hosts, whereas maize sites are located mostly in agricultural habitats where disturbance by human activity is constant, and the crop and most other vegetation dies at the end of the rainy season, so that overwintering hosts for corn leafhoppers are scarce (Medina et al. 2012).

Morphological divergence in wings between the teosinte- and maize-associated populations was strongly influenced by allometry, as indicated by the loss of host plant

effect after using size as an independent variable in the shape analysis. In forewings, overall host effect strength for both sexes was weak or moderate in the form analysis, and weaker still and non-significant in the shape analysis. This suggested that forewings vary in their shape almost exclusively dependent on body size changes. Size-dependent changes in organisms are a common, and well documented mechanism to maintain functionality (Strauss 1990; Sweet 1980). Although the nested effect of site within host for forewings was significant in form and shape—except in female forewing shape—, it was weak in magnitude, thus supporting the idea of allometry and not environment/habitat acting as a major factor for changes in forewing shape. In hindwings, the host plant effect on form was stronger for both sexes compared to forewings, but its strength was smaller almost by half, and the loss of significance in the shape analysis confirmed that allometry was the main source of morphological differentiation in hindwings between populations. This was also confirmed by the loss of significance for the nested effect in females. However, a moderate and significant effect of site within host was evident in the wing in shape analysis for males, thus suggesting that conditions other than host availability at the collecting sites are causing divergence in the hindwings of Perennial teosinte-associated compared to maize-associated and mixed-host sites specimens. For instance, Bernal et al. (in prep.) suggested that the isolation of Las Joyas site as consequence of its location within a nature reserve could enhance habitat isolation, thus contributing to the differentiation they found at the genotypic level. It would be worthwhile to test if that hypothesis extends to the morphological level by contrasting the environmental/habitat conditions present at Las

Joyas (e.g., temperature, rainfall, relative humidity, flora, other herbivores and natural enemies) versus the remaining collecting sites. However, the strong pattern of size-dependent morphological variation found in my study was not surprising as it may be a general biological phenomenon, and has been previously reported in other species (Strauss 1990; Sweet 1980). For example, Strauss (1990) showed that size accounted for 91% of morphological wing variation found in two butterfly groups in the family Nymphalidae (Lepidoptera).

Overall, my results showed that morphological differentiation is consistent with genetic structuring previously documented in corn leafhopper in western Mexico (Dávila-Flores 2012; Medina et al. 2012; Bernal et al. in prep), and suggested a strong effect of host plant and a moderate, but important, effect of environmental conditions at the collecting sites on the morphology of individuals. Particularly, Perennial teosinte-associated individuals were generally larger relative to maize-associated individuals, possibly as a consequence of environmental factors, such as elevation. Additionally, my results suggested that phenotypic differentiation is independent of genotypic differentiation, because while phenotypic differentiation is maintained, genetic differentiation was lost between the Perennial teosinte-only site and sites where maize and Perennial teosinte grow side by side. Thus, phenotypic plasticity may be a relevant factor shaping phenotype in corn leafhopper. Lastly, my results showed that variation in wing morphology is highly dependent on body size variation, but also suggested that environmental/habitat conditions in the teosinte-only site (e.g., favored by its locations within a nature reserve) may be acting as an incipient force leading to morphological

divergence in male hindwings. Further studies should focus on the implications of morphological divergence in dispersal patterns of corn leafhopper, as well as on quantitative genetic analyses to determine which environmental variables are selecting for population differentiation in morphology, and to what extent phenotypic plasticity is involved in differentiation.

CHAPTER IV

CONCLUSION

Overall, my study's result were inconsistent with the predicted trade-off between herbivore tolerance and resistance in plants (Agrawal et al. 1999; Fineblum and Rausher 1995; van der Meijden et al. 1988), and revealed a correlation between morphological differentiation and genetic differentiation reported earlier in corn leafhopper (Medina et al. 2012; Bernal et al. in prep.). The results of Chapter II suggested that Balsas teosinte, maize landraces, and maize inbred lines are similarly tolerant to the feeding damage by corn leafhopper at the seedling stage. I argued that this might be the result of different selection forces acting on tolerance and resistance, and suggested that future studies should examine metabolic processes such as respiration rate and photosynthate concentration/translocation in order to detect and understand the effects of phloem-feeders on plant tolerance variables other than regrowth. I also highlighted the importance of including females in further studies to determine whether oviposition constitutes a different type of injury or signal for the plant, which might interact with feeding and/or affect tolerance measured as regrowth. My study also pointed to the necessity of incorporating in future studies analyses at the biochemical level (e.g., defense metabolites and their phytohormone precursors) and phenological stages beyond the seedling stage, in order to couple that information with my results on tolerance and have a better understanding of the evolution of defense mechanisms in *Zea*. Concerning the domestication and breeding transitions, my results showed that although tolerance

was not mediated by those transitions, the architecture of *Zea* was mediated by domestication and breeding, and shoot: root ratios were mediated by domestication, with smaller shoot: root ratios in both maize landrace and maize inbred lines compared to Balsas teosinte, as expected in a process of directed selection for higher productivity.

My findings from Chapter III revealed consistency between morphological differentiation and genetic structuring previously reported in corn leafhopper in Mexico (Medina et al. 2012; Bernal et al. in prep.). My results showed that body size is the main morphological character segregating Perennial teosinte-associated population from maize-associated populations, with the former exhibiting generally larger dimensions. I suggested that phenotypic plasticity and environmental/habitat conditions in the collecting sites might be playing an important role in body size determination given the strong host effect and moderate, but important, effects of environmental conditions (e.g., elevation), as revealed by the estimates of effect strength. The strong host effect on corn leafhopper morphology I found contrasted with the loss of genetic structuring reported earlier between sites where maize and Perennial teosinte coexist versus sites where only Perennial teosinte grows (Bernal et al. in prep.). This suggested that different mechanisms mediate morphology and genotype. I also showed with my study that morphological variation in wings is highly dependent on body size, and that the moderate host effect I found might be leading not only to size- but to shape differentiation in male hindwings. I suggested that future studies should focus on quantitative genetics analyses of both populations to determine whether the proportion of

explained variation obtained with quantitative genetic methods is consistent with the strong host effect found in my study.

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